Baskar, P.
10/062624

(FILE 'HCAPLUS' ENTERED AT 10:32:25 ON 09 JUL 2003)

-key terms

L1 116 SEA FILE=HCAPLUS ABB=ON PLU=ON (EHRLICH? OR E)(W)CANIS

L2 89 SEA FILE=HCAPLUS ABB=ON PLU=ON L1 AND (EHRLICHIO#### OR CANINE OR DOG OR CANIS FAMILIAR?)

L3 18 SEA FILE=HCAPLUS ABB=ON PLU=ON L2 AND (28KD? OR 28KILOD? OR 28(W)(KD? OR KILOD? OR KILO(W)(D OR DA OR DALTON)) OR P28)

L1 116 SEA FILE=HCAPLUS ABB=ON PLU=ON (EHRLICH? OR E)(W)CANIS

L4 23 SEA FILE=HCAPLUS ABB=ON PLU=ON L1 AND (28KD? OR 28KILOD? OR 28(W)(KD? OR KILOD? OR KILO(W)(D OR DA OR DALTON)) OR P28)

L5 23 L3 OR L4

L5 ANSWER 1 OF 23 HCAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 2003:339170 HCAPLUS
DOCUMENT NUMBER: 138:367457
TITLE: Kinetics of antibody response to **Ehrlichia canis** immunoreactive proteins
AUTHOR(S): McBride, Jere W.; Corstvet, Richard E.; Gaunt, Steven D.; Boudreaux, Charles; Guedry, Thaya; Walker, David H.
CORPORATE SOURCE: Department of Pathology, University of Texas Medical Branch, Galveston, TX, 77555-0609, USA
SOURCE: Infection and Immunity (2003), 71(5), 2516-2524
CODEN: INFIBR; ISSN: 0019-9567
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Immunoreactive proteins of **Ehrlichia canis** and Ehrlichia chaffeensis that have been characterized include a family of **28-kDa** major outer membrane proteins (**p28**) and two large antigenically divergent surface glycoprotein orthologs. We previously demonstrated that recombinant **E. canis p28** and the 140- and 200-kDa glycoproteins gp140 and gp200, resp., react strongly with serum antibodies from suspect **canine ehrlichiosis** cases that were pos. for **E. canis** by immunofluorescent antibody test and in various phases of acute or chronic infection (2001). The kinetics of the antibody response to these potentially important vaccine and immunodiagnostic candidates is not known. Acute-phase serum antibody responses to whole-cell **E. canis** lysates and recombinant **p28**, gp140, and gp200 were monitored for 6 wk in **dogs** exptl. infected with **E. canis**. Irresp. of the inoculation route, a T-helper 1-type response was elicited to **E. canis** antigens consisting of IgG2 antibodies exclusively in both acute and convalescent phases in most **dogs**. Anal. of immunoreactive antigens for peak intensity and relative quantity identified major immunoreactive **E. canis** antigens recognized early in the infection as the 19-,

37-, 75-, and 140-kDa proteins. Later in infection, addnl. major immunoreactive **E. canis** proteins were identified, including the 28-, 47-, and 95-kDa proteins and the recently identified 200-kDa glycoprotein. All **dogs** had developed antibody against the recombinant gp140, gp200, and **p28** in the convalescent phase. Immunoreactivity and antibody response kinetics suggest that major immunoreactive proteins identified are immunodominant, but early recognition suggests increased dominance by some antigens.

REFERENCE COUNT: 48 THERE ARE 48 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 2 OF 23 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2002:966997 HCAPLUS
DOCUMENT NUMBER: 138:203265
TITLE: Investigation of **dog** monocytic **ehrlichiosis** by immunoblot analysis with **P28** protein of Ehrlichia chaffeensis
AUTHOR(S): Jian, Rui; Wen, Bohai; Pan, Hua; Liu, Shizhong
CORPORATE SOURCE: Department of Microbiology, Third Military Medical University, Chungking, 400038, Peop. Rep. China
SOURCE: Zhongguo Renshou Gonghuanbing Zazhi (Chinese Journal of Zoonoses) (2002), 18(4), 11-13
CODEN: ZRGZAP; ISSN: 1002-2694
PUBLISHER: Zhongguo Renshou Gonghuanbing Zazhi Bianweihui
DOCUMENT TYPE: Journal
LANGUAGE: Chinese
AB Aim: To investigate the epidemiol. of **dog** monocytic **ehrlichiosis** for better prevention and treatment of this disease. Methods: **Dog** sera were collected in southern China, where **Ehrlichia canis** infection had been identified, and used for immunoblotting with **P28** fusion protein of Ehrlichia chaffeensis as an antigen. Results: Eighty-nine out of 212 sera (42%) were pos., most of which were from the **dogs** working in fields, whereas the sera from the pet **dogs** were all neg. High level antibodies to **P28** were identified from month 4 to month 10, the active time for ticks in this area. Conclusion: **Dog** monocytic **ehrlichiosis** is an endemic disease in China, and the ticks are probably the vehicle of this disease.

L5 ANSWER 3 OF 23 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2002:609913 HCAPLUS
DOCUMENT NUMBER: 137:166520
TITLE: PCR primers and methods for detecting **Ehrlichia canis** and Ehrlichia chaffeensis in vertebrate and invertebrate hosts
INVENTOR(S): Stich, Roger William; Rikihisa, Yasuko
PATENT ASSIGNEE(S): The Ohio State University Research Foundation, USA
SOURCE: U.S., 36 pp.
CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 6432649	B1	20020813	US 2000-648520	20000825

PRIORITY APPLN. INFO.: US 2000-648520 20000825

AB Tools and methods for detecting the presence of **E. canis** and E. chaffeensis (the human granulocytic **ehrlichiosis** agent) in a sample obtained from an animal, such as human or **dogs**, are provided. The methods employ a polymerase chain reaction and primer sets that are based on the p30 gene of **E. canis** and the **p28** gene of E. chaffeensis. The present invention also relates to the p30 and the **p28** primer sets. Each p30 primer set comprises a first primer and the second primer, both of which are from 15 to 35 nucleotides in length. These primers are selected using criteria including annealing scores, identity of the primers to homologous E. chaffeensis sequences, and the availability of similarly optimal primers that are nested within the target template sequence. The methods are exemplified by detecting a 200bp-DNA fragment of **E. canis** p30 gene from the blood from **dog** carriers, or a 236bp-DNA fragment of E. chaffeensis **p28** gene from exptl. infected ticks of four species known to parasitize **dogs**. The p30-based assay is very sensitive than a previously reported nested 16S ribosomal DNA (rDNA)-based assay and only amplifies the 200-bp target amplicon from E. chaffeensis but not from Ehrlichia muris DNA. Optimized procedures for prepg. tissues from the infected hosts (**dog** carriers or infected ticks) and PCR conditions are described. The methods are useful for clin. diagnosis as well as exptl. investigations.

REFERENCE COUNT: 6 THERE ARE 6 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 4 OF 23 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2002:555769 HCAPLUS
DOCUMENT NUMBER: 137:124190
TITLE: Ehrlichia antigenic peptides for diagnosis of infections by **Ehrlichia canis** and Ehrlichia chaffeensis
INVENTOR(S): Lawton, Robert; O'Connor, Thomas Patrick, Jr.; Bartol, Barbara Ann; Machenry, Paul Scott
PATENT ASSIGNEE(S): Idexx Laboratories, Inc., USA
SOURCE: PCT Int. Appl., 29 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002057794	A2	20020725	WO 2002-US1395	20020116

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM,

AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

US 2002177178	A1	20021128	US 2001-765739	20010118
US 2002160432	A1	20021031	US 2002-54647	20020122
US 2003119082	A1	20030626	US 2002-54354	20020122

PRIORITY APPLN. INFO.: US 2001-765739 A 20010118

AB The invention provides methods and compns. for the detection of **Ehrlichia canis** and Ehrlichia chaffeensis antibodies and antibody fragments. The antigenic epitopes are identified using phage display technol. and are derived from **Ehrlichia canis** P30-1, P30, **P28**, OMP-1C, OMP-1D, OMP-1E, and OMP-1F. These antigenic epitope polypeptides are used in reversible flow chromatog. binding assay, ELISA, western blot, or indirect FIA for detecting the presence of antibodies or fragments to **Ehrlichia canis** and Ehrlichia chaffeensis.

L5 ANSWER 5 OF 23 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2002:444530 HCAPLUS
DOCUMENT NUMBER: 137:29031
TITLE: Protein and DNA sequences of **Ehrlichia canis** homologous **28-kilodalton** immunodominant protein gene family and uses thereof
INVENTOR(S): Walker, David H.; Yu, Xue-Jie; McBride, Jere W.
PATENT ASSIGNEE(S): Research Development Foundation, USA
SOURCE: U.S., 42 pp., Cont.-in-part of U.S. Ser. No. 201,458.
CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 4
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 6403780	B1	20020611	US 1999-261358	19990303
US 6458942	B1	20021001	US 1998-201458	19981130
WO 2000032745	A2	20000608	WO 1999-US28075	19991124

W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

AU 2000019234	A5	20000619	AU 2000-19234	19991124
BR 9916141	A	20011204	BR 1999-16141	19991124
US 6392023	B1	20020521	US 2000-660587	20000912
US 2002115840	A1	20020822	US 2002-62624	20020131
US 2003073095	A1	20030417	US 2002-62051	20020131
US 2003096250	A1	20030522	US 2002-62920	20020131

PRIORITY APPLN. INFO.: US 1998-201458 A2 19981130

US 1999-261358 A 19990303
WO 1999-US28075 W 19991124
US 2000-660279 A3 20000912
US 2000-660587 A3 20000912

AB The present invention is directed to the cloning, sequencing and expression of homologous immunoreactive **28-kDa** protein genes, ECa28-1, ECaSA2, and ECa28SA3, from a polymorphic multiple gene family of **Ehrlichia canis**. Further disclosed is a multigene locus encoding all five homologous **28-kDa** protein genes of **Ehrlichia canis**, and the five proteins are predicted to have signal peptides resulting in mature proteins and had amino acid homol. ranging from 51 to 72%. Anal. of intergenic regions revealed hypothetical promoter regions for each gene, suggesting that these genes may be independently and differentially expressed. The invention further provides expression vectors comprising genes encoding the **28-kDa** immunoreactive proteins and capable of expressing the gened when the vectors are introduced into cells. The invention discloses that the recombinant **Ehrlichia canis 28-kDa** proteins react with convalescent phase antiserum from an **E**. **canis**-infected **dog**.

REFERENCE COUNT: 1 THERE ARE 1 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 6 OF 23 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2002:387621 HCAPLUS
DOCUMENT NUMBER: 136:381390
TITLE: Protein and DNA sequences of homologous **28-kilodalton** immunodominant protein genes of **Ehrlichia canis** and therapeutical uses
INVENTOR(S): Walker, David H.; Yu, Xue-Jie; McBride, Jere W.
PATENT ASSIGNEE(S): Research Development Foundation, USA
SOURCE: U.S., 49 pp., Cont.-in-part of U.S. Ser. No. 261,358.
CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 4
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 6392023	B1	20020521	US 2000-660587	20000912
US 6458942	B1	20021001	US 1998-201458	19981130
US 6403780	B1	20020611	US 1999-261358	19990303
WO 2002022782	A2	20020321	WO 2001-US28759	20010912
WO 2002022782	A3	20020530		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH,

CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

AU 2001090926	A5	20020326	AU 2001-90926	20010912
EP 1317474	A2	20030611	EP 2001-970986	20010912
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				
US 2003073095	A1	20030417	US 2002-62051	20020131
US 2003096250	A1	20030522	US 2002-62920	20020131

PRIORITY APPLN. INFO.: US 1998-201458 A2 19981130
US 1999-261358 A2 19990303
US 2000-660587 A 20000912
WO 2001-US28759 W 20010912

AB The present invention is directed to the cloning, sequencing and expression of homologous immunoreactive **28-kDa** protein genes, **p28**-1, -2, -3, -5, -6, -7, -9, from a polymorphic multiple gene family of **Ehrlichia canis**. Further disclosed is a multigene locus encoding all nine homologous **28-kDa** protein genes of **Ehrlichia canis**. The invention also provides expression vectors comprising genes encoding the **28-kDa** proteins which are capable of expressing the recombinant proteins when the vectors are introduced into a cell. The **Ehrlichia canis 28-kDa** proteins react with convalescent phase antiserum from an **E. canis**-infected **dog**, and may be useful in the development of vaccines and serodiagnostics that are particularly effective for disease prevention and serodiagnosis.

REFERENCE COUNT: 3 THERE ARE 3 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 7 OF 23 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2002:220752 HCAPLUS
DOCUMENT NUMBER: 136:242995
TITLE: Homologous **28-kDa** immunodominant outer membrane protein genes of **Ehrlichia canis** and uses thereof for **dog** vaccine preparation to treat related infection
INVENTOR(S): Walker, David H.; Yu, Xue-Jie; Mcbride, Jere W.
PATENT ASSIGNEE(S): Research Development Foundation, USA
SOURCE: PCT Int. Appl., 106 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 4
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002022782	A2	20020321	WO 2001-US28759	20010912
WO 2002022782	A3	20020530		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT,

TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

US 6392023	B1	20020521	US 2000-660587	20000912
AU 2001090926	A5	20020326	AU 2001-90926	20010912
EP 1317474	A2	20030611	EP 2001-970986	20010912

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR

PRIORITY APPLN. INFO.:

US 2000-660587	A	20000912
US 1998-201458	A2	19981130
US 1999-261358	A2	19990303
WO 2001-US28759	W	20010912

AB The present invention is directed to the cloning, sequencing and expression of homologous immunoreactive **28-kDa** outer membrane protein genes, **p28**-1, -2, -3, -5, -6, -7, -9, from a polymorphic multiple gene family of **Ehrlichia canis**. Further disclosed is a multigene locus encoding all nine homologous **28-kDa** protein genes of **Ehrlichia canis**. Recombinant **Ehrlichia canis 28-kDa** proteins react with convalescent phase antiserum from an **E.canis** -infected **dog**, and may be useful in the development of vaccines and serodiagnostics that are particularly effective for disease prevention and serodiagnosis. The invention also relates to methods and compns. directed toward the prevention of **E. canis** infection of **dogs**.

L5 ANSWER 8 OF 23 HCAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER:	2002:195789 HCAPLUS
DOCUMENT NUMBER:	137:227067
TITLE:	Detection of **Ehrlichia canis** in **canine** carrier blood and in individual experimentally infected ticks with a p30-based PCR assay
AUTHOR(S):	Stich, Roger W.; Rikihisa, Yasuko; Ewing, S. A.; Needham, Glen R.; Grover, Debra L.; Jittapalapong, Sathaporn
CORPORATE SOURCE:	Department of Veterinary Preventive Medicine, The Ohio State University, Columbus, OH, 43210-1092, USA
SOURCE:	Journal of Clinical Microbiology (2002), 40(2), 540-546 CODEN: JCMIDW; ISSN: 0095-1137
PUBLISHER:	American Society for Microbiology
DOCUMENT TYPE:	Journal
LANGUAGE:	English

AB Detection of vector-borne pathogens is necessary for investigation of their assocn. with vertebrate and invertebrate hosts. The ability to detect Ehrlichia spp. within individual exptl. infected ticks would be valuable for studies to evaluate the relative competence of different vector species and transmission scenarios. The purpose of this study was to develop a sensitive PCR assay based on oligonucleotide sequences from the unique **Ehrlichia canis** gene, p30, to facilitate studies that require monitoring this pathogen in **canine** and tick hosts during

exptl. transmission. Homologous sequences for Ehrlichia chaffeensis **p28** were compared to sequences of primers derived from a sequence conserved among **E. canis** isolates. Criteria for primer selection included annealing scores, identity of the primers to homologous E. chaffeensis sequences, and the availability of similarly optimal primers that were nested within the target template sequence. The p30-based assay was at least 100-fold more sensitive than a previously reported nested 16S ribosomal DNA (rDNA)-based assay and did not amplify the 200-bp target amplicon from E. chaffeensis, the human granulocytic **ehrlichiosis** agent, or Ehrlichia muris DNA. The assay was used to detect **E. canis** in **canine** carrier blood and in exptl. infected Rhipicephalus sanguineus ticks. Optimized procedures for prepg. tissues from these hosts for PCR assay are described. Our results indicated that this p30-based PCR assay will be useful for exptl. investigations, that it has potential as a routine test, and that this approach to PCR assay design may be applicable to other pathogens that occur at low levels in affected hosts.

REFERENCE COUNT: 37 THERE ARE 37 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 9 OF 23 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2001:871449 HCAPLUS
DOCUMENT NUMBER: 137:74198
TITLE: Identification of a **p28** gene in Ehrlichia ewingii: Evaluation of gene for use as a target for a species-specific PCR diagnostic assay
AUTHOR(S): Gusa, Asiya A.; Buller, Richard S.; Storch, Gregory A.; Huycke, Mark M.; Machado, Linda J.; Slater, Leonard N.; Stockham, Steven L.; Massung, Robert F.
CORPORATE SOURCE: Division of Viral and Rickettsial Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, GA, 30333, USA
SOURCE: Journal of Clinical Microbiology (2001), 39(11), 3871-3876
CODEN: JCMIDW; ISSN: 0095-1137
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB PCR was used to amplify a 537-bp region of an Ehrlichia ewingii gene encoding a homolog of the **28-kDa** major antigenic protein (**P28**) of Ehrlichia chaffeensis. The E. ewingii **p28** gene homolog was amplified from DNA extd. from whole blood obtained from four humans and one **canine** with confirmed cases of infection. Sequencing of the PCR products (505 bp) revealed a partial gene with homol. to outer membrane protein genes from Ehrlichia and Cowdria spp: p30 of **Ehrlichia canis** (.ltoreq.71.3%), **p28** of E. chaffeensis (.ltoreq.68.3%), and map 1 of Cowdria ruminantium (67.3%). The peptide sequence of the E. ewingii partial gene product was deduced (168 amino acids) and the antigenicity profile was analyzed, revealing a hydrophilic protein with .ltoreq.69.1% identity to **P28** of E. chaffeensis, .ltoreq.67.3% identity to P30 of

E. canis, and .ltoreq.63.1% identity to MAP1 of C. ruminantium. Primers were selected from the E. ewingii **p28** sequence and used to develop a species-specific PCR diagnostic assay. The **p28** PCR assay amplified the expected 215-bp product from DNA that was extd. from EDTA-treated blood from each of the confirmed E. ewingii infections that were available. The assay did not produce PCR products with DNA extd. from E. chaffeensis-, **E. canis**-, or E. phagocytophila-infected samples, confirming the specificity of the **p28** assay for E. ewingii. The sensitivity of the E. ewingii-specific PCR assay was evaluated and detd. to detect as few as 38 copies of the **p28** gene.

REFERENCE COUNT: 28 THERE ARE 28 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 10 OF 23 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2001:816394 HCAPLUS
DOCUMENT NUMBER: 135:356748
TITLE: P43 antigen for the immunodiagnosis of **canine ehrlichiosis** and uses thereof
INVENTOR(S): Walker, David H.; McBride, Jere W.
PATENT ASSIGNEE(S): Research Development Foundation, USA
SOURCE: PCT Int. Appl., 60 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001082862	A2	20011108	WO 2001-US13446	20010427
WO 2001082862	A3	20020404		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
US 6355777	B1	20020312	US 2000-561322	20000428
AU 2001055702	A5	20011112	AU 2001-55702	20010427
EP 1276492	A2	20030122	EP 2001-928896	20010427
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				

PRIORITY APPLN. INFO.: US 2000-561322 A 20000428
WO 2001-US13446 W 20010427

AB **Canine** monocytic **ehrlichiosis**, caused by **Ehrlichia canis** is a potentially fatal disease of **dogs** that requires rapid and accurate diagnosis in order to initiate appropriate therapy leading to a favorable prognosis. In the invention described herein, a new immunoreactive **E.**

canis surface protein gene of 1170-bp was cloned, which encodes a protein with a predicted mol. mass of 42.6 kilodaltons (P43). The P43 gene was not found in E. chaffeensis DNA by Southern blot, and antisera against recombinant P43 (rP43) did not react with E. chaffeensis by indirect fluorescent antibody (IFA). The P43 was located on the surface of **E. canis** by immunoelectron microscopy. Forty-two **dogs** exhibiting signs and/or hematol. abnormalities assocd. with **canine ehrlichiosis** were tested by IFA and by Western immunoblot. Among the 22 samples that were IFA pos. for **E. canis**, 100 reacted with the rP43, 96 with the rP28, and 96 with the rP140. The specificity of the recombinant proteins compared to IFA was 96 for rp28, 88 for P43 and 63 for P140. Results of this study demonstrate that the rP43 and rP28 are sensitive and reliable serodiagnostic antigens for the diagnosis of **Ehrlichia canis** infections.

L5 ANSWER 11 OF 23 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2001:473743 HCAPLUS
DOCUMENT NUMBER: 138:118175
TITLE: Analysis of transcriptionally active gene clusters of major outer membrane protein multigene family in **Ehrlichia canis** and E. chaffeensis. [Erratum to document cited in CA135:340042]
AUTHOR(S): Ohashi, Norio; Rikihisa, Yasuko; Unver, Ahmet
CORPORATE SOURCE: Department of Veterinary Biosciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH, 43210-1093, USA
SOURCE: Infection and Immunity (2001), 69(7), 4702
CODEN: INFIBR; ISSN: 0019-9567
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB On page 2083, abstr., line 2, "in humans and **dogs**, resp." should read "in **dogs** and humans, resp.".

L5 ANSWER 12 OF 23 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2001:240579 HCAPLUS
DOCUMENT NUMBER: 135:340042
TITLE: Analysis of transcriptionally active gene clusters of major outer membrane protein multigene family in **Ehrlichia canis** and E. chaffeensis
AUTHOR(S): Ohashi, Norio; Rikihisa, Yasuko; Unver, Ahmet
CORPORATE SOURCE: Department of Veterinary Biosciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH, 43210-1093, USA
SOURCE: Infection and Immunity (2001), 69(4), 2083-2091
CODEN: INFIBR; ISSN: 0019-9567
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB **Ehrlichia canis** and E. chaffeensis are tick-borne obligatory intramonocytic ehrlichiae that cause febrile systemic illness in **dogs** and humans, resp. The current study analyzed the pleomorphic multigene family encoding approx. 30-kDa major outer membrane proteins (OMPs) of **E.**

canis and E. chaffeensis. Upstream from secA and downstream of hypothetical transcriptional regulator, 22 paralogs of the omp gene family were found to be tandemly arranged except for one or two genes with opposite orientations in a 28- and a 27-kb locus in the **E. canis** and E. chaffeensis genomes, resp. Each locus consisted of three highly repetitive regions with four nonrepetitive intervening regions. **E. canis**, in addn., had a 6.9-kb locus which contained a repeat of three tandem paralogs in the 28-kb locus. These total 47 paralogous and orthologous genes encoded OMPs of approx. 30 to 35 kDa consisting of several hypervariable regions alternating with conserved regions. In the 5' -end half of the 27-kb locus or the 28-kb locus of each Ehrlichia species, 14 paralogs were linked by short intergenic spaces ranging from -8 bp (overlapped) to 27 bp, and 8 remaining paralogs in the 3' -end half were connected by longer intergenic spaces ranging from 213 to 632 bp. All 22 paralogs, five unknown genes, and secA in the omp cluster in **E. canis** were transcriptionally active in the monocyte culture, and the paralogs with short intergenic spaces were cotranscribed with their adjacent genes, including the resp. intergenic spaces at both the 5' and the 3' sides. Although omp genes are diverse, our results suggest that the gene organization of the clusters and the gene locus are conserved between two species of Ehrlichia to maintain a unique transcriptional mechanism for adaptation to environmental changes common to them.

REFERENCE COUNT: 33 THERE ARE 33 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 13 OF 23 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2001:83487 HCAPLUS
DOCUMENT NUMBER: 134:350187
TITLE: Immunodiagnosis of **Ehrlichia canis** infection with recombinant proteins
AUTHOR(S): McBride, Jere W.; Corstvet, Richard E.; Breitschwerdt, Edward B.; Walker, David H.
CORPORATE SOURCE: Department of Pathology and WHO Collaborating Center for Tropical Diseases, University of Texas Medical Branch, Galveston, TX, 77555, USA
SOURCE: Journal of Clinical Microbiology (2001), 39(1), 315-322
CODEN: JCMIDW; ISSN: 0095-1137
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB **Ehrlichia canis** causes a potentially fatal rickettsial disease of **dogs** that requires rapid and accurate diagnosis in order to initiate appropriate therapy leading to a favorable prognosis. We recently reported the cloning of two immunoreactive **E. canis** proteins, **P28** and P140, that were applicable for serodiagnosis of the disease. In the present study we cloned a new immunoreactive **E. canis** surface protein gene of 1,170 bp, which encodes a protein with a predicted mol. mass of 42.6 kDa (P43). The P43 gene was not detected in E. chaffeensis DNA by Southern blot, and antisera against recombinant P43 (rP43) did not react with E. chaffeensis as detected by indirect fluorescent antibody (IFA)

assay. Forty-two **dogs** exhibiting signs and/or hematol. abnormalities assocd. with **canine ehrlichiosis** were tested by IFA assay and by recombinant Western immunoblot. Among the 22 samples that were IFA pos. for **E. canis**, 100% reacted with rP43, 96% reacted with rP28, and 96% reacted with rP140. The specificity of the recombinant proteins compared to the IFAs was 96% for rP28, 88% for P43 and 63% for P140. The results of this study demonstrate that the rP43 and rP28 are sensitive and reliable serodiagnostic antigens for **E. canis** infections.

REFERENCE COUNT: 30 THERE ARE 30 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 14 OF 23 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2000:615928 HCAPLUS
DOCUMENT NUMBER: 134:81644
TITLE: A conserved, transcriptionally active **p28** multigene locus of **Ehrlichia canis**
AUTHOR(S): McBride, J. W.; Yu, X.-j.; Walker, D. H.
CORPORATE SOURCE: Department of Pathology and WHO Collaborating Center for Tropical Diseases, University of Texas Medical Branch, Galveston, TX, 77555-0609, USA
SOURCE: Gene (2000), 254(1,2), 245-252
CODEN: GENED6; ISSN: 0378-1119
PUBLISHER: Elsevier Science B.V.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Antigenic diversity of Ehrlichia chaffeensis and **Ehrlichia canis** may involve independent or differential expression of the **P28** outer membrane proteins genes, enabling persistent infections of the natural hosts. In this study, we analyzed the transcriptional activity of a five gene locus in **E. canis** encoding homologous, but non-identical, **p28** genes. The **p28** multigene locus contained three previously identified complete gene sequences and one partial gene sequence. A new **p28** gene was identified and sequenced, and the complete sequence of a second partial **p28** gene was detd. The new **p28** gene joined two previously sep. loci, forming the single **p28** multigene locus. The amino acid homol. of the **E. canis P28** proteins ranged from 51 to 74%. The nucleic acid sequence of regions compared within the locus spanning four **p28** genes from two geog. distinct **E. canis** isolates was completely conserved. Anal. of the five **p28** genes demonstrated that all were transcriptionally active in in-vitro cultures of **E. canis** incubated at the vertebrate host (37.degree.C) and ambient tick temps. (27.degree.C). Polycistronic copies of multiple **p28** genes were not detected by RT-PCR, but monocistronic **p28** mRNA transcripts were detected by Northern blotting from **E. canis** infected DH82 cells, indicating that the genes are transcribed as monocistronic messages.

REFERENCE COUNT: 22 THERE ARE 22 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 15 OF 23 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2000:384370 HCAPLUS
DOCUMENT NUMBER: 133:27381
TITLE: Sequences of two novel homologous **28-kilodalton** immunodominant protein genes (ECa28-1 and ECa28SA3) of **Ehrlichia canis** and uses thereof
INVENTOR(S): Walker, David H.; Yu, Xue-jie; McBride, Jere W.
PATENT ASSIGNEE(S): Research Development Foundation, USA
SOURCE: PCT Int. Appl., 86 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 4
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000032745	A2	20000608	WO 1999-US28075	19991124
W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
US 6458942	B1	20021001	US 1998-201458	19981130
US 6403780	B1	20020611	US 1999-261358	19990303
AU 2000019234	A5	20000619	AU 2000-19234	19991124
BR 9916141	A	20011204	BR 1999-16141	19991124

PRIORITY APPLN. INFO.:
US 1998-201458 A 19981130
US 1999-261358 A 19990303
WO 1999-US28075 W 19991124

AB The invention provides sequences of two novel homologous immunoreactive **28-kDa** protein genes, ECa28-1 and ECa28SA3, from a polymorphic multiple gene family of **Ehrlichia canis**. A complete sequence of another **28-kDa** protein gene, ECa28SA2, which was previously only partially sequenced, is also provided. Further disclosed is a multigene locus (5.592-kb) encoding all five homologous **28-kDa** outer membrane protein genes (ECa28SA1, ECa28SA2, ECa28SA3, ECa28-1, and ECa28-2). Recombinant **Ehrlichia canis 28-kDa** proteins react with convalescent phase antiserum from an **E. canis**-infected **dog**. The invention also relates to methods and compns. directed toward the prevention of **E. canis** infection of **dogs**.

L5 ANSWER 16 OF 23 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1999:809483 HCAPLUS
DOCUMENT NUMBER: 132:277873
TITLE: Western and dot blotting analyses of Ehrlichia chaffeensis indirect fluorescent-antibody assay-positive and -negative human sera by using native and recombinant E. chaffeensis and **E. canis** antigens

AUTHOR(S): Unver, Ahmet; Rikihisa, Yasuko; Ohashi, Norio; Cullman, Louis C.; Buller, Richard; Storch, Gregory A.
CORPORATE SOURCE: Department of Veterinary Biosciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH, 43210-1093, USA
SOURCE: Journal of Clinical Microbiology (1999), 37(12), 3888-3895
CODEN: JCMIDW; ISSN: 0095-1137
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Human monocytic **ehrlichiosis** is an emerging infectious disease caused by Ehrlichia chaffeensis, a gram-neg. obligatory intracellular bacterium closely related to **E. canis**. The immunoreactive recombinant fusion proteins rP28 and rP30 have become available after cloning and expressing of the 28- and 30-kDa major outer membrane protein genes of E. chaffeensis and **E. canis**, resp. Western immunoblotting was performed to analyze the antibody responses of the 37 E. chaffeensis indirect fluorescent-antibody assay (IFA)-pos. and 20 IFA-neg. serum specimens with purified whole organisms, rP28, and rP30. All IFA-neg. sera were neg. with purified whole organisms, rP28, or rP30 by Western immunoblot anal. (100% relative diagnostic specificity). Of 37 IFA-pos. sera, 34 sera reacted with any native proteins of E. chaffeensis ranging from 44 to 110 kDa, and 30 sera reacted with 44- to 110-kDa native **E. canis** antigens. The **28-kDa** E. chaffeensis and 30-kDa **E. canis** native proteins were recognized by 25 IFA-pos. sera. Fifteen IFA-pos. sera reacted with rP28 by Western blot anal., whereas 34 IFA-pos. sera reacted with rP30 (92% relative diagnostic specificity), indicating that rP30 is more sensitive than rP28 for detecting the antibodies in IFA-pos. sera. These 34 IFA-pos. sera were pos. by the dot blot assay with rP30, distinguishing them from IFA-neg. sera. Except for three rP30-neg. but IFA-pos. specimens that instead showed an E. ewingii infection-like profile by Western immunoblotting, the results of Western and dot blot assays with rP30 matched 100% with the IFA test results. Densitometric anal. of dot blot reactions showed a pos. correlation between the dot d. and the IFA titer. These results suggest that rP30 antigen would provide a simple, consistent, and rapid serodiagnosis for human monocytic **ehrlichiosis**.
REFERENCE COUNT: 35 THERE ARE 35 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 17 OF 23 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1999:515994 HCAPLUS
DOCUMENT NUMBER: 131:285238
TITLE: Variability in the **28-kDa** Surface Antigen Protein Multigene Locus of Isolates of the Emerging Disease Agent Ehrlichia chaffeensis Suggests That It Plays a Role in Immune Evasion
AUTHOR(S): Reddy, Ganta Roman; Streck, Christopher P.
CORPORATE SOURCE: Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS, 66506, USA

SOURCE: Molecular Cell Biology Research Communication (1999), 1(3), 167-175
CODEN: MCBCFS; ISSN: 1522-4724
PUBLISHER: Academic Press
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Infections caused by rickettsial pathogens persist in vertebrate hosts for long periods of time, despite the active host immune response. The authors recently described the multigene locus encoding **28 kDa** surface antigen proteins (**28 kDa** SAPs) for two closely related rickettsials, Ehrlichia chaffeensis and **Ehrlichia canis**, that share extensive structural homol. to antigenic variant surface protein genes of Neisseria and Borrelia species. In this study, the authors describe motifs for recombinase binding sites and a high frequency of repeat elements in the cloned **28 kDa** SAP genes. The locus for two newly established E. chaffeensis isolates also was characterized, and immunol. specificity of the **28 kDa** SAPs was investigated. The study identified variant forms of the **28 kDa** SAPs and extensive restriction fragment length polymorphisms among isolates. The mol. data suggest that the locus is influenced by short-term evolutionary changes such as genetic recombinations leading to the generation of antigenic variants. Antigenic variation could contribute to the mechanism of immune evasion and the emergence of new diseases. (c) 1999 Academic Press.

REFERENCE COUNT: 31 THERE ARE 31 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 18 OF 23 HCAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 1999:336049 HCAPLUS
DOCUMENT NUMBER: 131:165989

TITLE: Molecular cloning of the gene for a conserved major immunoreactive **28-kilodalton** protein of **Ehrlichia canis**: a potential serodiagnostic antigen

AUTHOR(S): McBride, Jere W.; Yu, Xue-Jie; Walker, David H.
CORPORATE SOURCE: Department of Pathology and WHO Collaborating Center for Tropical Diseases, University of Texas Medical Branch, Galveston, TX, 77555-0609, USA

SOURCE: Clinical and Diagnostic Laboratory Immunology (1999), 6(3), 392-399
CODEN: CDIMEN; ISSN: 1071-412X
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A gene encoding a **28-kDa** protein of **Ehrlichia canis** was cloned, sequenced, and expressed, and a comparative mol. anal. with homologous genes of **E. canis**, Cowdria ruminantium, and Ehrlichia chaffeensis was performed. The complete gene has an 834-bp open reading frame encoding a protein of 278 amino acids with a predicted mol. mass of 30.5 kDa. An N-terminal signal sequence was identified, suggesting that the protein undergoes posttranslational modification to a mature 27.7-kDa protein (**P28**). The

E. canis p28 gene has significant nucleic acid and amino acid sequence homologies with the E. chaffeensis outer membrane protein-1 (omp-1) gene family, with the Cowdria ruminantium map-1 gene, and with other **E. canis 28-kDa**-protein genes. Southern blotting revealed the presence of at least two addnl. homologous **p28** gene copies in the **E. canis** genome, confirming that **p28** is a member of a polymorphic multiple-gene family. Amino acid sequence anal. revealed that **E. canis P28** has four variable regions, and it shares similar surface-exposed regions, antigenicity, and T-cell motifs with E. chaffeensis **P28**. The **p28** genes from seven different **E. canis** isolates were identical, indicating that the gene for this major immunoreactive protein is highly conserved. In addn., reactivity of sera from clin. cases of **canine ehrlichiosis** with the recombinant **P28** demonstrated that the recombinant protein may be a reliable serodiagnostic antigen.

REFERENCE COUNT: 33 THERE ARE 33 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 19 OF 23 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1998:578729 HCAPLUS
DOCUMENT NUMBER: 130:33659
TITLE: Cloning and characterization of multigenes encoding the immunodominant 30-kilodalton major outer membrane proteins of **Ehrlichia canis** and application of the recombinant protein for serodiagnosis
AUTHOR(S): Ohashi, Norio; Unver, Ahmet; Zhi, Ning; Rikihisa, Yasuko
CORPORATE SOURCE: Department of Veterinary Biosciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH, 43210-1093, USA
SOURCE: Journal of Clinical Microbiology (1998), 36(9), 2671-2680
CODEN: JCMIDW; ISSN: 0095-1137
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A 30-kDa major outer membrane protein of **Ehrlichia canis**, the agent of **canine ehrlichiosis**, is the major antigen recognized by both naturally and exptl. infected **dog** sera. The protein cross-reacts with a serum against a recombinant **28-kDa** protein (rP28), one of the outer membrane proteins of a gene (omp-1) family of Ehrlichia chaffeensis. Two DNA fragments of **E. canis** were amplified by PCR with two primer pairs based on the sequences of E. chaffeensis omp-1 genes, cloned, and sequenced. Each fragment contained a partial 30-kDa protein gene of **E. canis**. Genomic Southern blot anal. with the partial gene probes revealed the presence of multiple copies of these genes in the **E. canis** genome. Three copies of the entire gene (p30, p30-1, and p30a) were cloned and sequenced from the **E. canis** genomic DNA. The open reading frames of the two copies (p30 and p30-1) were tandemly arranged with an intergenic space. The three copies were similar but not identical

and contained a semivariable region and three hypervariable regions in the protein mols. The following genes homologous to three **E. canis** 30-kDa protein genes and the E. chaffeensis omp-1 family were identified in the closely related rickettsiae: wsp from Wolbachia sp., p44 from the agent of human granulocytic **ehrlichiosis**, msp-2 and msp-4 from Anaplasma marginale, and map-1 from Cowdria ruminantium. Phylogenetic anal. among the three **E. canis** 30-kDa proteins and the major surface proteins of the rickettsiae revealed that these proteins are divided into four clusters and the two **E. canis** 30-kDa proteins are closely related but that the third 30-kDa protein is not. The p30 gene was expressed as a fusion protein, and the antibody to the recombinant protein (rP30) was raised in a mouse. The antibody reacted with rP30 and a 30-kDa protein of purified **E. canis**. Twenty-nine indirect fluorescent antibody (IFA)-pos. **dog** plasma specimens strongly recognized the rP30 of **E. canis** . To evaluate whether the rP30 is a suitable antigen for serodiagnosis of **canine ehrlichiosis**, the immunoreactions between rP30 and the whole purified **E. canis** antigen were compared in the dot immunoblot assay. Dot reactions of both antigens with IFA-pos. **dog** plasma specimens were clearly distinguishable by the naked eye from those with IFA-neg. plasma specimens. By densitometry with a total of 42 IFA-pos. and -neg. plasma specimens, both antigens produced results similar in sensitivity and specificity. These findings suggest that the rP30 antigen provides a simple, consistent, and rapid serodiagnosis for **canine ehrlichiosis**. Cloning of multigenes encoding the 30-kDa major outer membrane proteins of **E. canis** will greatly facilitate understanding pathogenesis and immunol. study of **canine** ehrlichosis and provide a useful tool for phylogenetic anal.

REFERENCE COUNT: 34 THERE ARE 34 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 20 OF 23 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1998:429259 HCAPLUS
DOCUMENT NUMBER: 129:171273
TITLE: Molecular characterization of a **28 kDa** surface antigen gene family of the tribe Ehrlichiae
AUTHOR(S): Reddy, G. Roman; Sulsona, Carlos R.; Barbet, Anthony F.; Mahan, Suman M.; Burridge, Michael J.; Alleman, Arthur R.
CORPORATE SOURCE: Dep. Pathobiol., Univ. Florida, Gainesville, FL, 32610, USA
SOURCE: Biochemical and Biophysical Research Communications (1998), 247(3), 636-643
CODEN: BBRCA9; ISSN: 0006-291X
PUBLISHER: Academic Press
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Antisera against different Ehrlichiae recognize an immunodominant, cross-reacting .apprx.**28 kDa** surface antigen defined as the MAP1 in Cowdria ruminantium. These antigens are considered valuable in developing serodiagnostic tests and recombinant vaccines for Ehrlichiae infections. To evaluate the

relationship in three closely related Ehrlichiae, Ehrlichiae chaffeensis, **Ehrlichiae canis**, and C. ruminantium, the structure of the **28 kDa** antigen genes was analyzed. We describe the cloning and characterization of DNA encoding genes homologous to MAP1 from E. chaffeensis and **E. canis**. The cloned segment of E. chaffeensis contains one expressed and four transcriptionally silent tandemly arranged, nonidentical genes; the **E. canis** locus consists of two nonidentical genes. Comparative anal. of these genes revealed the presence of four conserved regions sepd. by three highly variable regions. B-cell epitope anal. identified three major cross-reacting epitopes that map to the variable regions. Location of the epitopes at the variable regions and the presence of multigene family with only one expressed copy suggest a mechanism of immune evasion in these Ehrlichiae. (c) 1998 Academic Press.

REFERENCE COUNT: 38 THERE ARE 38 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 21 OF 23 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1998:7815 HCAPLUS
DOCUMENT NUMBER: 128:163375
TITLE: Immunodominant major outer membrane proteins of Ehrlichia chaffeensis are encoded by a polymorphic multigene family
AUTHOR(S): Ohashi, Norio; Zhi, Ning; Zhang, Yilan; Rikihisa, Yasuko
CORPORATE SOURCE: Department of Veterinary Biosciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH, 43210-1093, USA
SOURCE: Infection and Immunity (1998), 66(1), 132-139
CODEN: INFIBR; ISSN: 0019-9567
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Several immunodominant major proteins ranging from 23 to 30 kDa were identified in the outer membrane fractions of Ehrlichia chaffeensis and **Ehrlichia canis**. The N-terminal amino acid sequence of a **28-kDa** protein of E. chaffeensis (one of the major proteins) was detd. The gene (**p28**), almost full length, encoding the **28-kDa** protein was cloned by PCR with primers designed based on the N-terminal sequence of the E. chaffeensis **28-kDa** protein and the consensus sequence between the C termini of the Cowdria ruminantium MAP-1 and Anaplasma marginale MSP-4 proteins. The **p28** gene was overexpressed, and antibody to the recombinant protein was raised in a rabbit. The antibody and serum from a patient infected with E. chaffeensis reacted with the recombinant protein, three proteins (29, 28, and 25 kDa) of E. chaffeensis, and a 30-kDa protein of **E. canis**. Immunoelectron microscopy with the rabbit antibody revealed that the antigenic epitope of the **28-kDa** protein was exposed on the surface of E. chaffeensis. Southern blot anal. with a 32P-labeled **p28** gene probe revealed multiple copies of genes homologous to **p28** in the E. chaffeensis genome. Six copies of the **p28** gene were cloned and sequenced from the genomic DNA by using the same probe. The open reading frames of these gene copies were tandemly arranged with intergenic spaces. They were

nonidentical genes and contained a semivariable region and three hypervariable regions in the predicted protein mols. One of the gene copies encoded a protein with an internal amino acid sequence identical to the chem. detd. N-terminal amino acid sequence of a 23-kDa protein of E. chaffeensis. Immunization with the recombinant **P28** protein protected mice from infection with E. chaffeensis. These findings suggest that the 30-kDa-range proteins of E. chaffeensis represent a family of antigenically related homologous proteins encoded by a single gene family.

REFERENCE COUNT: 44 THERE ARE 44 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 22 OF 23 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1997:767892 HCAPLUS
DOCUMENT NUMBER: 128:33472
TITLE: Western immunoblotting analysis of the antibody responses of patients with human monocytotropic **ehrlichiosis** to different strains of Ehrlichia chaffeensis and **Ehrlichia canis**
AUTHOR(S): Chen, Sheng-Min; Cullman, Louis C.; Walker, David H.
CORPORATE SOURCE: Department of Pathology, University of Texas Medical Branch, Galveston, TX, 77555-0609, USA
SOURCE: Clinical and Diagnostic Laboratory Immunology (1997), 4(6), 731-735
CODEN: CDIMEN; ISSN: 1071-412X
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB To evaluate the relative sensitivity of the detection of antibodies against various antigenic proteins of E. chaffeensis for the diagnosis of the emerging infectious disease human monocytotropic **ehrlichiosis**, Western immunoblotting was performed with 27 serum samples from convalescent patients with antibodies, as demonstrated by indirect immunofluorescence assay. Among 22 patients with antibodies reactive with the 120-kDa protein, 15 showed reactivity with the 29/**28-kDa** protein(s) and the proteins in the 44-88-kDa range. Two of the serum samples with this pattern reacted with the 29/**28-kDa** protein(s) of only the 91HE17 strain, and one sample reacted with only that of the Arkansas strain, indicating that the antibodies were stimulated by strain-specific epitopes. Overall, antibodies to the 29/**28-kDa** protein(s) were detected in only 16 patients' sera, suggesting that this protein is less sensitive than the 120-kDa protein. Two of 12 serum samples from healthy blood donors had antibodies reactive with the 120-kDa protein; one of these samples reacted also with the 29/**28-kDa** protein(s) of **E. canis**, suggesting that unrecognized ehrlichial infection might have occurred, including human infection with **E. canis**. A high correlation between reactivity with the 120-kDa protein by Western immunoblotting and the recombinant 120-kDa protein by dot blot supports the potential usefulness of this recombinant antigen in diagnostic serol.

L5 ANSWER 23 OF 23 HCAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 1996:305975 HCAPLUS
DOCUMENT NUMBER: 125:29500
TITLE: Analysis and ultrastructural localization of Ehrlichia chaffeensis proteins with monoclonal antibodies
AUTHOR(S): Chen, Sheng-Min; Popov, Vsevolod L.; Feng, Hui-Min; Walker, David H.
CORPORATE SOURCE: Department Pathology, University Texas Medical Branch, Galveston, TX, USA
SOURCE: American Journal of Tropical Medicine and Hygiene (1996), 54(4), 405-412
CODEN: AJTHAB; ISSN: 0002-9637
PUBLISHER: American Society of Tropical Medicine and Hygiene
DOCUMENT TYPE: Journal
LANGUAGE: English

AB To det. the nature and ultrastructural location of E. chaffeensis antigens, monoclonal antibodies (MAbs) to E. chaffeensis were developed. The MAbs were used for immunofluorescence and Western immunoblotting anal. of the antigens of d. gradient-purified ehrlichiae. Monoclonal antibody 6A1 recognized an epitope of a 30-kD protein. This antibody reacted with a strain-specific epitope of E. chaffeensis, Arkansas strain, and did not cross-react with any other ehrlichia tested. Monoclonal antibodies 3C7 and 7C1-B recognized Arkansas strain proteins of 30 and 29 kD and reacted with E. chaffeensis (strain 91HE17) proteins of 31 and 29 kD and an **E. canis** protein of 30 kD. Lack of reactivity of these 2 MAbs with E. sennetsu and E. risticii suggests that the epitope is group specific. Monoclonal antibody 5D11 recognized a 58-kD protein of both strains of E. chaffeensis as well as **E. canis**, apparently a group-specific, conformation-independent epitope. Monoclonal antibody 7C1-C reacted with 58- and 88-kD proteins of both the Arkansas and 91HE17 strains. Trypsin treatment destroyed the reactivity of E. chaffeensis antigens with all the MAbs when tested by Western immunoblotting, indicating that these antigens are proteins with trypsin-sensitive epitopes. Immunoelectron microscopy of neg. stained intact E. chaffeensis organisms showed that the 30- and 29-kD proteins are present on the surface of the ehrlichial cell wall along with the previously localized **28-kD** protein.

(FILE 'MEDLINE, BIOSIS, EMBASE, WPIDS, CONFSCI, SCISEARCH, JICST-EPLUS, JAPIO, CABA, AGRICOLA, VETU, VETB' ENTERED AT 10:48:40 ON 09 JUL 2003)

L6 64 S L3
L7 83 S L4
L8 83 S L6 OR L7
L9 31 DUP REM L8 (52 DUPLICATES REMOVED)

L9 ANSWER 1 OF 31 MEDLINE DUPLICATE 1
ACCESSION NUMBER: 2003200429 MEDLINE
DOCUMENT NUMBER: 22590245 PubMed ID: 12704123
TITLE: Kinetics of antibody response to **Ehrlichia canis** immunoreactive proteins.
AUTHOR: McBride Jere W; Corstvet Richard E; Gaunt Steven D; Boudreaux Charles; Guedry Thaya; Walker David H
CORPORATE SOURCE: Department of Pathology, Sealy Center for Vaccine Development, and Center for Biodefense and Emerging

Infectious Diseases, University of Texas Medical Branch, Galveston, Texas 77555-0609, USA..
jemcbride@utmb.edu
SOURCE: INFECTION AND IMMUNITY, (2003 May) 71 (5) 2516-24.
Journal code: 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200305
ENTRY DATE: Entered STN: 20030501
Last Updated on STN: 20030516
Entered Medline: 20030515

AB Immunoreactive proteins of **Ehrlichia canis** and Ehrlichia chaffeensis that have been characterized include a family of **28-kDa** major outer membrane proteins (**p28**) and two large antigenically divergent surface glycoprotein orthologs. We previously demonstrated that recombinant **E. canis p28** and the 140- and 200-kDa glycoproteins gp140 and gp200, respectively, react strongly with serum antibodies from suspect **canine ehrlichiosis** cases that were positive for **E. canis** by immunofluorescent antibody test and in various phases of acute or chronic infection (J. Clin. Microbiol. 39:315-322, 2001). The kinetics of the antibody response to these potentially important vaccine and immunodiagnostic candidates is not known. Acute-phase serum antibody responses to whole-cell **E. canis** lysates and recombinant **p28**, gp140, and gp200 were monitored for 6 weeks in **dogs** experimentally infected with **E. canis**. Irrespective of the inoculation route, a T-helper 1-type response was elicited to **E. canis** antigens consisting of immunoglobulin G2 antibodies exclusively in both acute and convalescent phases in most **dogs**. Analysis of immuoreactive antigens for peak intensity and relative quantity identified major immunoreactive **E. canis** antigens recognized early in the infection as the 19-, 37-, 75-, and 140-kDa proteins. Later in infection, additional major immunoreactive **E. canis** proteins were identified, including the 28-, 47-, and 95-kDa proteins and the recently identified 200-kDa glycoprotein. All **dogs** had developed antibody against the recombinant gp140, gp200, and **p28** in the convalescent phase. Immunoreactivity and antibody response kinetics suggest that major immunoreactive proteins identified are immunodominant, but early recognition suggests increased dominance by some antigens.

L9 ANSWER 2 OF 31 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2002:609790 BIOSIS
DOCUMENT NUMBER: PREV200200609790
TITLE: **28-kDa** immunoreactive protein gene of **Ehrlichia canis** and uses thereof.
AUTHOR(S): Walker, David H.; McBride, Jere W. (1); Yu, Xue-Jie
CORPORATE SOURCE: (1) Galveston, TX USA
ASSIGNEE: Research Development Foundation, Alexandria, VA, USA
PATENT INFORMATION: US 6458942 October 01, 2002
SOURCE: Official Gazette of the United States Patent and

Trademark Office Patents, (Oct. 1, 2002) Vol. 1263, No. 1, pp. No Pagination. http://www.uspto.gov/web/menu/patdata.html. e-file. ISSN: 0098-1133.
DOCUMENT TYPE: Patent
LANGUAGE: English
AB The present invention is directed to the cloning, sequencing and expression of a conserved immunoreactive **28-kDa** protein gene (**P28**) from a polymorphic multiple gene family of **Ehrlichia canis**. **E. canis** **P28** has an 834-bp open reading frame encoding a protein of 278 amino acids with four variable regions, and shares similar surface-exposed regions, antigenicity and T-cell motifs with E. chaffeensis **P28**. Also disclosed is that recombinant **E. canis P28** protein reacts with convalescent phase antiserum from an **E. canis** -infected **dog**.

L9 ANSWER 3 OF 31 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2002:501879 BIOSIS
DOCUMENT NUMBER: PREV200200501879
TITLE: Methods for detecting **Ehrlichia** **canis** and Ehrlichia chaffeensis in vertebrate and invertebrate hosts.
AUTHOR(S): Stich, Roger William (1); Rikihisa, Yasuko
CORPORATE SOURCE: (1) Columbus, OH USA
ASSIGNEE: The Ohio State University Research Foundation
PATENT INFORMATION: US 6432649 August 13, 2002
SOURCE: Official Gazette of the United States Patent and Trademark Office Patents, (Aug. 13, 2002) Vol. 1261, No. 2, pp. No Pagination. http://www.uspto.gov/web/menu/patdata.html. e-file. ISSN: 0098-1133.
DOCUMENT TYPE: Patent
LANGUAGE: English
AB Tools and methods for detecting the presence of **E.** **canis** and E. chaffeensis in a sample obtained from an animal are provided. The methods employ a polymerase chain reaction and primer sets that are based on the p30 gene of **E.** **canis** and the **p28** gene of E. chaffeensis. The present invention also relates to the p30 and the **p28** primer sets. Each p30 primer set comprises a first primer and the second primer, both of which are from 15 to 35 nucleotides in length. The first p30 primer comprises a sequence which is complementary to a consecutive sequence, within the following sequence: CCA AGTGTCTCAC ATTTTGGTAG CTTCTCAGCT AAAGAAGAAA GCAAATCAAC TGTTGGAGTTTTTGGATTAA AACATGATTG GGATGGAAGT CCAATACTTA AGAATAAACA CGCTGACTTTACTGTTCCAA AC. SEQ ID NO.1. The second p30 primer comprises a sequence which is complementary to the inverse complement of a consecutive sequence contained within the following sequence: GTTACT CAATGGGTGG CCCAAGAATA GAATTCGAAA TATCTTATGA AGCATTCGAC GTAAAAAGTC CTAATATCAA TTATCAAAAT GACGCGCACA GGTACTGCGC TCTATCTCAT CACACATCGG CAGCCAT, SEQ ID NO.2. The first **p28** comprises a sequence which is complementary to a consecutive sequenc, within the following sequence : A GTTTTCATAA CAAGTGCATT GATATCACTA ATATCTTCTC TACCTGGAGT ATCATTTTCC GACCCAACAG GTAGTGGTAT TAACGG, SEQ ID NO. 3. The second **p28** primer comprises a

sequence which is complementary to the inverse complement of a consecutive sequence within one of the following two sequences: CAT TTCTAGGTTT TGCAGGAGCT ATTGGCTACT CAATGGATGG TCCAAGAATA GAGCTTGAAG TATCTTATGA, SEQ ID NO. 4, or C AAGGAAAGTT AGGTTTAAGC TACTCTATAA GCCCAGA, SEQ ID NO. 5.

L9 ANSWER 4 OF 31 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2002:400661 BIOSIS
DOCUMENT NUMBER: PREV200200400661
TITLE: Homologous **28-kilodalton** immunodominant protein genes of **ehrlichia canis** and uses thereof.
AUTHOR(S): Walker, David H.; Yu, Xue-Jie; McBride, Jere W.
ASSIGNEE: Research Development Foundation
PATENT INFORMATION: US 6403780 June 11, 2002
SOURCE: Official Gazette of the United States Patent and Trademark Office Patents, (June 11, 2002) Vol. 1259, No. 2, pp. No Pagination. http://www.uspto.gov/web/menu/patdata.html. e-file. ISSN: 0098-1133.
DOCUMENT TYPE: Patent
LANGUAGE: English
AB The present invention is directed to the cloning, sequencing and expression of homologous immunoreactive **28-kDa** protein genes, ECa28-1 and ECa28SA3, from a polymorphic multiple gene family of **Ehrlichia canis**. A complete sequence of another **28-kDa** protein gene, ECaSA2, is also provided. Further disclosed is a multigene locus encoding all five homologous **28-kDa** protein genes of **Ehrlichia canis**. Recombinant **Ehrlichia canis 28-kDa** proteins react with convalescent phase antiserum from an **E. canis** -infected **dog**.

L9 ANSWER 5 OF 31 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2002:348237 BIOSIS
DOCUMENT NUMBER: PREV200200348237
TITLE: Homologous **28-kilodalton** immunodominant protein genes of **Ehrlicha canis** and uses thereof.
AUTHOR(S): Walker, David H.; Yu, Xue-Jie (1); McBride, Jere W.
CORPORATE SOURCE: (1) Houston, TX USA
ASSIGNEE: Research Development Foundation
PATENT INFORMATION: US 6392023 May 21, 2002
SOURCE: Official Gazette of the United States Patent and Trademark Office Patents, (May 21, 2002) Vol. 1258, No. 3, pp. No Pagination. http://www.uspto.gov/web/menu/patdata.html. e-file. ISSN: 0098-1133.
DOCUMENT TYPE: Patent
LANGUAGE: English
AB The present invention is directed to the cloning, sequencing and expression of homologous immunoreactive **28-kDa** protein genes, **p28**-1, -2, -3, -5, -6, -7, -9, from a polymorphic multiple gene family of **Ehrlichia canis**. Further disclosed is a multigene locus encoding all nine homologous **28-kDa** protein genes of **Ehrlichia canis**. Recombinant **Ehrlichia**

canis 28-kDa proteins react with convalescent phase antiserum from an **E. canis** -infected **dog**, and may be useful in the development of vaccines and serodiagnostics that are particularly effective for disease prevention and serodiagnosis.

L9 ANSWER 6 OF 31 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2002:278689 BIOSIS
DOCUMENT NUMBER: PREV200200278689
TITLE: P43 antigen for the immunodiagnosis of **canine ehrlichiosis** and uses thereof.
AUTHOR(S): Walker, David H. (1); McBride, Jere W.
CORPORATE SOURCE: (1) Galveston, TX USA
ASSIGNEE: Research Development Foundation
PATENT INFORMATION: US 6355777 March 12, 2002
SOURCE: Official Gazette of the United States Patent and Trademark Office Patents, (Mar. 12, 2002) Vol. 1256, No. 2, pp. No Pagination. http://www.uspto.gov/web/menu/patdata.html. e-file. ISSN: 0098-1133.
DOCUMENT TYPE: Patent
LANGUAGE: English
AB **Canine** monocytic **ehrlichiosis**, caused by Ehrilichia canis is a potentially fatal disease of **dogs** that requires rapid and accurate diagnosis in order to initiate appropriate therapy leading to a favorable prognosis. In the invention described herein, a new immunoreactive **E. canis** surface protein gene of 1170-bp was cloned, which encodes a protein with a predicted molecular mass of 42.6 kilodaltons (P43). The P43 gene was not found in E. chaffeensis DNA by Southern blot, and antisera against recombinant P43 (rP43) did not react with E. chaffeensis by IFA. The P43 was located on the surface of **E. canis** by immunoelectron microscopy. Forty-two **dogs** exhibiting signs and/or hematologic abnormalities associated with **canine ehrlichiosis** were tested by IFA and by Western immunoblot. Among the 22 samples that were IFA positive for **E. canis**, 100% reacted with the rP43, 96% with the rP28, and 96% with the rP140. The specificity of the recombinant proteins compared to IFA was 96% for rP28, 88% for P43 and 63% for P140. Results of this study demonstrate that the rP43 and rP28 are sensitive and reliable serodiagnostic antigens for the diagnosis of **Ehrlichia canis** infections.

L9 ANSWER 7 OF 31 WPIDS (C) 2003 THOMSON DERWENT
ACCESSION NUMBER: 2002-351882 [38] WPIDS
CROSS REFERENCE: 2000-412298 [35]
DOC. NO. CPI: C2002-099984
TITLE: New recombinant homologous **28 kilodalton** immunodominant protein from **Ehrlichia canis**, useful for treating **Ehrlichia canis** infections.
DERWENT CLASS: B04 C06 D16
INVENTOR(S): MCBRIDE, J W; WALKER, D H; YU, X
PATENT ASSIGNEE(S): (RERE-N) RES DEV FOUND; (MCBR-I) MCBRIDE J W; (WALK-I) WALKER D H; (YUXX-I) YU X
COUNTRY COUNT: 96

PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2002022782	A2	20020321	(200238)*	EN	106
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZW					
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG UZ VN YU ZA ZW					
US 6392023	B1	20020521	(200239)		
AU 2001090926	A	20020326	(200251)		
US 2002115840	A1	20020822	(200258)		
US 2003073095	A1	20030417	(200329)		
US 2003096250	A1	20030522	(200336)		
EP 1317474	A2	20030611	(200339)	EN	
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI TR					

APPLICATION DETAILS:

PATENT NO	KIND		APPLICATION	DATE
WO 2002022782	A2		WO 2001-US28759	20010912
US 6392023	B1	CIP of	US 1998-201458	19981130
		CIP of	US 1999-261358	19990303
			US 2000-660587	20000912
AU 2001090926	A		AU 2001-90926	20010912
US 2002115840	A1	CIP of	US 1998-201458	19981130
		CIP of	US 1999-261358	19990303
		Div ex	US 2000-660587	20000912
			US 2002-62624	20020131
US 2003073095	A1	CIP of	US 1998-201458	19981130
		CIP of	US 1999-261358	19990303
		Div ex	US 2000-660587	20000912
			US 2002-62051	20020131
US 2003096250	A1	CIP of	US 1998-201458	19981130
		CIP of	US 1999-261358	19990303
		Div ex	US 2000-660587	20000912
			US 2002-62920	20020131
EP 1317474	A2		EP 2001-970986	20010912
			WO 2001-US28759	20010912

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 2001090926	A	Based on	WO 200222782
US 2002115840	A1	CIP of	US 6403780
US 2003073095	A1	Div ex	US 6392023
		CIP of	US 6403780
		CIP of	US 6458942
US 2003096250	A1	Div ex	US 6392023
		CIP of	US 6403780
		CIP of	US 6458942
EP 1317474	A2	Based on	WO 200222782

PRIORITY APPLN. INFO: US 2000-660587 20000912; US 1998-201458 19981130; US 1999-261358 19990303; US 2002-62624 20020131; US 2002-62051 20020131; US 2002-62920 20020131

AN 2002-351882 [38] WPIDS

CR 2000-412298 [35]

AB WO 200222782 A UPAB: 20030619

NOVELTY - A recombinant homologous **28 kDa** immunodominant protein (I), of **Ehrlichia canis** comprising a sequence (S1) of 278, 283, 280, 293, 276 or 271 amino acids, given in the specification, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) an isolated DNA sequence (II) encoding a 30 kDa protein of **E. canis**, selected from **p28**-1,-2,-3,-6,-7 and **p28**-9, where the protein is immunoreactive with anti-**E. canis** serum;

(2) a vector (III) comprising (II);

(3) a host cell (IV) comprising a nucleic acid segment comprising a sequence (S2) of 1607, 849, 840, 879, 840, 828 or 813 nucleotides, given in the specification;

(4) producing (I) comprising:

(a) obtaining a vector comprising an expression region comprising a sequence encoding S1, operatively linked to a promoter;

(b) transfecting the vector into a cell; and

(c) culturing the cell under conditions effective for expression of the expression region; and

(5) an antibody (Ab) immunoreactive with (I); and

(6) inhibiting **E. canis** infection in a subject comprising identifying a subject prior to exposure or suspected of being to exposed to or infected with **E. canis**, and administering a composition comprising (I).

ACTIVITY - Antibacterial.

MECHANISM OF ACTION - Vaccine. No biological data is given.

USE - (I), a **28-kDa** antigen preferably dispersed in a pharmaceutically acceptable carrier, is useful for inhibiting **E. canis** infection in a subject (claimed). (I) is useful in the development of vaccines and serodiagnostics that are particularly effective for disease prevention and serodiagnosis.

Dwg.0/16

L9 ANSWER 8 OF 31 MEDLINE DUPLICATE 2

ACCESSION NUMBER: 2002116442 MEDLINE

DOCUMENT NUMBER: 21683633 PubMed ID: 11825969

TITLE: Detection of **Ehrlichia canis** in **canine** carrier blood and in individual experimentally infected ticks with a p30-based PCR assay.

AUTHOR: Stich Roger W; Rikihisa Yasuko; Ewing S A; Needham Glen R; Grover Debra L; Jittapalapong Sathaporn

CORPORATE SOURCE: Department of Veterinary Preventive Medicine, The Ohio State University, Columbus, Ohio 43210-1092, USA.. stich.2@osu.edu

SOURCE: JOURNAL OF CLINICAL MICROBIOLOGY, (2002 Feb) 40 (2) 540-6.
Journal code: 7505564. ISSN: 0095-1137.

PUB. COUNTRY: United States
DOCUMENT TYPE: (EVALUATION STUDIES)
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200205
ENTRY DATE: Entered STN: 20020220
Last Updated on STN: 20020602
Entered Medline: 20020531

AB Detection of vector-borne pathogens is necessary for investigation of their association with vertebrate and invertebrate hosts. The ability to detect Ehrlichia spp. within individual experimentally infected ticks would be valuable for studies to evaluate the relative competence of different vector species and transmission scenarios. The purpose of this study was to develop a sensitive PCR assay based on oligonucleotide sequences from the unique **Ehrlichia canis** gene, p30, to facilitate studies that require monitoring this pathogen in **canine** and tick hosts during experimental transmission. Homologous sequences for Ehrlichia chaffeensis **p28** were compared to sequences of primers derived from a sequence conserved among **E. canis** isolates. Criteria for primer selection included annealing scores, identity of the primers to homologous E. chaffeensis sequences, and the availability of similarly optimal primers that were nested within the target template sequence. The p30-based assay was at least 100-fold more sensitive than a previously reported nested 16S ribosomal DNA (rDNA)-based assay and did not amplify the 200-bp target amplicon from E. chaffeensis, the human granulocytic **ehrlichiosis** agent, or Ehrlichia muris DNA. The assay was used to detect **E. canis** in **canine** carrier blood and in experimentally infected Rhipicephalus sanguineus ticks. Optimized procedures for preparing tissues from these hosts for PCR assay are described. Our results indicated that this p30-based PCR assay will be useful for experimental investigations, that it has potential as a routine test, and that this approach to PCR assay design may be applicable to other pathogens that occur at low levels in affected hosts.

L9 ANSWER 9 OF 31 CABA COPYRIGHT 2003 CABI
ACCESSION NUMBER: 2002:155908 CABA
DOCUMENT NUMBER: 20023116254
TITLE: Investigation of **dog** monocytic **ehrlichiosis** by immunoblot analysis with **P28** protein of Ehrlichial chaffeensis
AUTHOR: Jian Rui; Wen BoHai; Pan Hua; Liu ShiZhong; Jian, R.; Wen, B. H.; Pan, H.; Liu, S. Z.
CORPORATE SOURCE: Department of Microbiology, Third Military Medical University, Chongqing 400038, China.
SOURCE: Chinese Journal of Zoonoses, (2002) Vol. 18, No. 4, pp. 11-13. 10 ref.
ISSN: 1002-2694
DOCUMENT TYPE: Journal
LANGUAGE: Chinese
SUMMARY LANGUAGE: English

AB **Dog** monocytic **ehrlichiosis** was investigated to provide information for preventing and treating this disease. **Dog** sera were collected in an area of southern China in

which **E. canis**-infection was identified; the sera were examined by immunoblot using **P28** fusion protein of E. chaffeensis as an antigen. 89 of 212 sera (42%) were positive in immunoblot analysis and most of the positive sera from the **dogs** working and training in fields, but the sera from the pet **dogs** were all negative. The high level of antibodies to **P28** was from month 4 to month 10, which corresponded to the time of tick activity in this area. **Dog** monocytic **ehrlichiosis** is an endemic disease in China, the ticks are the vehicle of this disease.

L9 ANSWER 10 OF 31 WPIDS (C) 2003 THOMSON DERWENT
ACCESSION NUMBER: 2002-049309 [06] WPIDS
DOC. NO. CPI: C2002-013857
TITLE: New isolated and purified **Ehrlichia canis** immunoreactive surface protein, P43, for use as an antigen in immunodiagnosis of **canine ehrlichiosis**, and for preparation of a vaccine against **canine ehrlichiosis**.
DERWENT CLASS: B04 C06 D16
INVENTOR(S): MCBRIDE, J W; WALKER, D H
PATENT ASSIGNEE(S): (RERE-N) RES DEV FOUND
COUNTRY COUNT: 96
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2001082862	A2	20011108	(200206)*	EN	60
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZW					
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG UZ VN YU ZA ZW					
US 6355777	B1	20020312	(200221)		
AU 2001055702	A	20011112	(200222)		
EP 1276492	A2	20030122	(200308)	EN	
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI TR					

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2001082862	A2	WO 2001-US13446	20010427
US 6355777	B1	US 2000-561322	20000428
AU 2001055702	A	AU 2001-55702	20010427
EP 1276492	A2	EP 2001-928896	20010427
		WO 2001-US13446	20010427

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 2001055702	A	Based on	WO 200182862
EP 1276492	A2	Based on	WO 200182862

PRIORITY APPLN. INFO: US 2000-561322 20000428
AN 2002-049309 [06] WPIDS
AB WO 200182862 A UPAB: 20020128
NOVELTY - An isolated and purified **Ehrlichia canis** immunoreactive surface protein, P43, (I) with a predicted molecular mass of 42.6 kilodaltons, coded by an isolated DNA selected from:
(a) a DNA which encodes (I);
(b) a DNA which hybridizes to (a); and
(c) a DNA differing from (a) or (b) in codon sequence due to the degeneracy of the genetic code, is new.
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:
(1) a DNA (II) which encodes (I) selected from:
(a) an isolated DNA which encodes (I);
(b) an isolated DNA which hybridizes to (a) and encodes (I); and
(c) an isolated DNA differing from (a) or (b) in codon sequence due to the degeneracy of the genetic code, and which encodes (I);
(2) a vector (III) comprising (II) and regulatory elements necessary for expression of (II) in a cell;
(3) a host cell (IV) transfected with (III) which expresses (I);
(4) an antibody (V) directed against (I);
(5) a vaccine (VI) against **canine ehrlichiosis** comprising (I);
(6) determining (M) whether a **dog** is infected with **E. canis**, involves (M1) determining whether serum from the **dog** reacts with (I), or (M2) extracting DNA from the blood of the **dog**, performing a polymerase chain reaction (PCR) amplification on the DNA with oligonucleotide primers specific for (II), and separating the resulting PCR product by size, where positive detection of an appropriately sized amplification product indicates **E. canis** infection;
(7) a serodiagnostic kit (VII) for performing (M1), comprising:
(a) immobilized **E. canis** antigens selected from P43 protein, **P28** protein and both P43 and **P28** protein;
(b) dilution buffers for **dog** serum;
(c) an anti-**dog** serum second antibody linked to a reporter molecule; and
(d) reagents for detection of the reporter molecule; and
(8) a kit (VIII) for performing (M2), comprising reagents for DNA extraction from blood, p43-specific oligonucleotides, and reagents for PCR amplification.
ACTIVITY - Acaricide.
MECHANISM OF ACTION - Vaccine (claimed). No biological data is given.
USE - (I) is useful as an antigen in the immunodiagnosis of **canine ehrlichiosis**, and for the preparation of vaccine against **canine ehrlichiosis**.
ADVANTAGE - (I) is highly immunoreactive, and is sensitive and reliable for the serologic diagnosis of **canine** monocytotrophic **ehrlichiosis**. Serodiagnosis of **canine ehrlichiosis** was performed by indirect fluorescent-antibody (IFA) and recombinant proteins. Forty-two cases clinically suspected to be **canine ehrlichiosis** were taken, and when evaluated by IFA, 22 seropositive cases with

titers ranging from 40 to greater than 40960 were detected. Approximately half of the 42 samples had titers greater than 80, and the other half had titers of 40 or less, which provided the appropriate samples for evaluation of overall sensitivity of the IFA and recombinant proteins. Twenty of the 42 samples were negative by IFA at 1:40. The recombinant **Ehrlichia canis** immunoreactive surface protein (rP43) had the best correlation with positive IFA samples at 100 % sensitivity, followed by rP28 (96 %) and the rP140 (96 %). All samples with IFA titers of 80 had 100 % positive correlation with all of the recombinant antigens, and the density of the reaction by Western immunoblot appeared to be proportional to the IFA titer. The rP43 and rP28 exhibited the best combination of sensitivity and specificity, and the rP140 reacted non-specifically with several IFA negative sera. The observation that three **dogs** which were IFA negative for **E. canis** were weakly positive to the rP43 antigen, suggested that this antigen was more sensitive than the IFA, rather than less specific.
Dwg.0/7

L9 ANSWER 11 OF 31 MEDLINE DUPLICATE 3
ACCESSION NUMBER: 2001574839 MEDLINE
DOCUMENT NUMBER: 21538942 PubMed ID: 11682500
TITLE: Identification of a **p28** gene in Ehrlichia ewingii: evaluation of gene for use as a target for a species-specific PCR diagnostic assay.
AUTHOR: Gusa A A; Buller R S; Storch G A; Huycke M M; Machado L J; Slater L N; Stockham S L; Massung R F
CORPORATE SOURCE: Division of Viral and Rickettsial Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia 30333, USA.
SOURCE: JOURNAL OF CLINICAL MICROBIOLOGY, (2001 Nov) 39 (11) 3871-6.
Journal code: 7505564. ISSN: 0095-1137.
PUB. COUNTRY: United States
DOCUMENT TYPE: (EVALUATION STUDIES)
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200203
ENTRY DATE: Entered STN: 20011030
Last Updated on STN: 20020314
Entered Medline: 20020313

AB PCR was used to amplify a 537-bp region of an Ehrlichia ewingii gene encoding a homologue of the **28-kDa** major antigenic protein (**P28**) of Ehrlichia chaffeensis. The E. ewingii **p28** gene homologue was amplified from DNA extracted from whole blood obtained from four humans and one **canine** with confirmed cases of infection. Sequencing of the PCR products (505 bp) revealed a partial gene with homology to outer membrane protein genes from Ehrlichia and Cowdria spp.: p30 of **Ehrlichia canis** (< or =71.3%), **p28** of E. chaffeensis (< or =68.3%), and map1 of Cowdria ruminantium (67.3%). The peptide sequence of the E. ewingii partial gene product was deduced (168 amino acids) and the antigenicity profile was analyzed, revealing a hydrophilic protein with < or =69.1% identity to **P28** of E. chaffeensis, < or =67.3% identity to P30 of **E. canis**, and < or =63.1% identity to MAP1 of C.

ruminantium. Primers were selected from the E. ewingii **p28** sequence and used to develop a species-specific PCR diagnostic assay. The **p28** PCR assay amplified the expected 215-bp product from DNA that was extracted from EDTA-treated blood from each of the confirmed E. ewingii infections that were available. The assay did not produce PCR products with DNA extracted from E. chaffeensis-, **E. canis**-, or E. phagocytophila-infected samples, confirming the specificity of the **p28** assay for E. ewingii. The sensitivity of the E. ewingii-specific PCR assay was evaluated and determined to detect as few as 38 copies of the **p28** gene.

L9 ANSWER 12 OF 31 MEDLINE DUPLICATE 4
ACCESSION NUMBER: 2001550448 MEDLINE
DOCUMENT NUMBER: 21480270 PubMed ID: 11596732
TITLE: Serologic and molecular evidence of coinfection with multiple vector-borne pathogens in **dogs** from Thailand.
AUTHOR: Suksawat J; Xuejie Y; Hancock S I; Hegarty B C; Nilkumhang P; Breitschwerdt E B
CORPORATE SOURCE: Department of Veterinary Medicine, Faculty of Veterinary Medicine, Khon Kaen University, Thailand.
SOURCE: JOURNAL OF VETERINARY INTERNAL MEDICINE, (2001 Sep-Oct) 15 (5) 453-62.
Journal code: 8708660. ISSN: 0891-6640.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AF082744; GENBANK-M83801; GENBANK-U26740
ENTRY MONTH: 200202
ENTRY DATE: Entered STN: 20011015
Last Updated on STN: 20020222
Entered Medline: 20020221

AB Forty-nine **dogs** from Thailand were evaluated for serologic evidence of exposure or polymerase chain reaction (PCR) evidence of infection with vectorborne pathogens, including Ehrlichia sp. (**Ehrlichia canis**, Ehrlichia chaffeensis, Ehrlichia equi, and Ehrlichia risticii), Bartonella vinsonii subsp. berkhoffi (Bvb), spotted fever group (SFG) rickettsiae (Rickettsia rickettsii), Typhus group (TG) rickettsiae (Rickettsia canada, Rickettsia prowazekii, and Rickettsia typhi), and Babesia sp. (Babesia canis and Babesia gibsonii). All study **dogs** had at least 1 of 3 entry criteria: fever, anemia, or thrombocytopenia. By immunofluorescence antibody (IFA) testing, seroreactivity was most prevalent to E chaffeensis (74%) and **E canis** (71%) antigens, followed by E equi (58%), Bvb (38%), E risticii (38%), R prowazekii (24%), B canis (20%), R rickettsii (12%), R canada (4%), and B gibsonii (4%) antigens. There was 100% concordance between **E canis** IFA and Western blot immunoassay (WI) for 35 of 35 samples; 2 samples were IFA and WI reactive only to E equi antigens. By PCR amplification, 10 **dogs** were found to be infected with **E canis**, 5 with Ehrlichia platys, and 3 with B canis. Sequencing of PCR products was undertaken to compare Ehrlichia strains from Thailand to strains originating from the United States. Partial DNA sequence analysis confirmed infection with **E canis** and E platys, with identical 16S rRNA sequence

alignment to **E canis** (U26740) and to E platys (M83801), as reported in GenBank. Partial **E canis** **P28**.1 and **P28**.2 amino acid sequences from Thai **dogs** were divergent from analogous sequences derived from North American **E canis** (AF082744) strains, suggesting that the Thai **dogs** were infected with a geographically distinct strain of **E canis** compared to North American strains. The results of this study indicate that **dogs** in Thailand have substantial exposure to vectorborne diseases and that coinfection with these pathogens may be common.

L9 ANSWER 13 OF 31 MEDLINE DUPLICATE 5
ACCESSION NUMBER: 2001131092 MEDLINE
DOCUMENT NUMBER: 20579049 PubMed ID: 11136790
TITLE: Immunodiagnosis of **Ehrlichia canis** infection with recombinant proteins.
AUTHOR: McBride J W; Corstvet R E; Breitschwerdt E B; Walker D H
CORPORATE SOURCE: Department of Pathology and WHO Collaborating Center for Tropical Diseases, University of Texas Medical Branch, Galveston, Texas 77555, USA.
CONTRACT NUMBER: AI31431 (NIAID)
SOURCE: JOURNAL OF CLINICAL MICROBIOLOGY, (2001 Jan) 39 (1) 315-22.
Journal code: 7505564. ISSN: 0095-1137.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AF252298
ENTRY MONTH: 200103
ENTRY DATE: Entered STN: 20010404
Last Updated on STN: 20010404
Entered Medline: 20010301

AB **Ehrlichia canis** causes a potentially fatal rickettsial disease of **dogs** that requires rapid and accurate diagnosis in order to initiate appropriate therapy leading to a favorable prognosis. We recently reported the cloning of two immunoreactive **E. canis** proteins, **P28** and P140, that were applicable for serodiagnosis of the disease. In the present study we cloned a new immunoreactive **E. canis** surface protein gene of 1,170 bp, which encodes a protein with a predicted molecular mass of 42.6 kDa (P43). The P43 gene was not detected in E. chaffeensis DNA by Southern blot, and antisera against recombinant P43 (rP43) did not react with E. chaffeensis as detected by indirect fluorescent antibody (IFA) assay. Forty-two **dogs** exhibiting signs and/or hematologic abnormalities associated with **canine ehrlichiosis** were tested by IFA assay and by recombinant Western immunoblot. Among the 22 samples that were IFA positive for **E. canis**, 100% reacted with rP43, 96% reacted with rP28, and 96% reacted with rP140. The specificity of the recombinant proteins compared to the IFAs was 96% for rP28, 88% for P43 and 63% for P140. The results of this study demonstrate that the rP43 and rP28 are sensitive and reliable serodiagnostic antigens for **E. canis** infections.

L9 ANSWER 14 OF 31 VETU COPYRIGHT 2003 THOMSON DERWENT
ACCESSION NUMBER: 2001-62955 VETU
TITLE: Efficacy of an amitraz-impregnated collar in preventing transmission of Borrelia burgdorferi by adult Ixodes scapularis to **dogs**.
AUTHOR: Elfassy O J; Goodman F W; Levy S A; Carter L L
CORPORATE SOURCE: Virbac; Stillmeadow
LOCATION: Fort Worth; Sugar Land, Tex.; Durham, Conn., USA
SOURCE: J.Am.Vet.Med.Assoc. (219, No. 2, 185-89, 2001) 2 Fig. 25 Ref.
CODEN: JAVMA4
AVAIL. OF DOC.: Virbac AH Inc., 3200 Meacham Boulevard, Fort Worth, TX 76137, U.S.A. (F.W.G.).
LANGUAGE: English
DOCUMENT TYPE: Journal
FIELD AVAIL.: AB; LA; CT
AN 2001-62955 VETU
AB Ixodes scapularis is the vector of Borrelia burgborferi in the NE USA. In **dogs**, amitraz-impregnated collars (Preventic, Virbac) prevented the development of antibodies to B. burgdorferi after experimental infestation with I. scapularis naturally infected with B. burgdorferi. Amitraz-impregnated collars may be useful to prevent borreliosis in **dogs**.
ABEX 8 SPF Beagle **dogs** (6 or more mth-old, 7.9-14.4 kg, 5 male) were fitted with collars impregnated with amitraz or not treated, on day 0 of an 84 day study. On day 7 all were infested with 100 adult I. scapularis, of which 39.4% were naturally infected with B. burgdorferi; ticks were removed on day 17. Serum was taken on days 21, 28, 35, 42, 56, 70 and 84. Serum from the 4 treated **dogs** remained negative for antibodies against B. burgdorferi antigens by ELISA and Western blotting. By day 28, 3/4 controls were seropositive by ELISA; all controls developed strong antibody responses during the study. Control serum Western blotting showed bands typical of B. burgdorferi infection from day 28. By day 56 control serum showed distinct bands at MW consistent with B. burgdorferi antigens p19, p22, **p28**, p30 complex, p39 and others. All **dogs** were negative for antibodies to Babesia canis, **Ehrlichia canis** and Rickettsia rickettsii.

L9 ANSWER 15 OF 31 CABA COPYRIGHT 2003 CABI
ACCESSION NUMBER: 2002:158289 CABA
DOCUMENT NUMBER: 20013175016
TITLE: Diagnosis of **canine ehrlichiosis** by immunoblot with **P28** fusion protein of Ehrlichia chaffeensis
AUTHOR: Jian Rui; Wen BoHai; Fang YuQiang; Pan Hua; Liu ShiZhong; Jian, R.; Wen, B. H.; Fang, Y. Q.; Pan, H.; Liu, S. Z.
CORPORATE SOURCE: Department of Microbiology, The Third Military Medical University, Chongqing 400038, China.
SOURCE: Chinese Journal of Zoonoses, (2001) Vol. 17, No. 6, pp. 11-13. 11 ref.
ISSN: 1002-2694
DOCUMENT TYPE: Journal
LANGUAGE: Chinese
SUMMARY LANGUAGE: English

AB The diagnosis of **Ehrlichia canis** infections by immunoblot using the **P28** fusion protein of Ehrlichia chaffeensis is described. Positive results were observed in 3 sera from infected **dogs** and negative results were obtained in sera from normal **dogs**; 43 of 165 serum samples (26%) collected in an **E. canis** endemic area [China] were positive by immunoblotting.

L9 ANSWER 16 OF 31 WPIDS (C) 2003 THOMSON DERWENT
ACCESSION NUMBER: 2000-412298 [35] WPIDS
CROSS REFERENCE: 2002-351882 [38]
DOC. NO. CPI: C2000-125025
TITLE: **Ehrlichia canis** antigens useful for vaccinating against **canine ehrlichiosis** in **dogs**.
DERWENT CLASS: B04 C06 D16
INVENTOR(S): MCBRIDE, J W; WALKER, D H; YU, X
PATENT ASSIGNEE(S): (RERE-N) RES DEV FOUND
COUNTRY COUNT: 87
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2000032745	A2	20000608	(200035)*	EN	75
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ TZ UG ZW					
W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG UZ VN YU ZA ZW					
AU 2000019234	A	20000619	(200044)		
BR 9916141	A	20011204	(200203)		
KR 2001093122	A	20011027	(200223)		
US 6403780	B1	20020611	(200244)		
US 6458942	B1	20021001	(200268)		

APPLICATION DETAILS:

PATENT NO	KIND		APPLICATION	DATE
WO 2000032745	A2		WO 1999-US28075	19991124
AU 2000019234	A		AU 2000-19234	19991124
BR 9916141	A		BR 1999-16141	19991124
			WO 1999-US28075	19991124
KR 2001093122	A		KR 2001-706690	20010529
US 6403780	B1	CIP of	US 1998-201458	19981130
			US 1999-261358	19990303
US 6458942	B1		US 1998-201458	19981130

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 2000019234	A	Based on	WO 200032745
BR 9916141	A	Based on	WO 200032745

PRIORITY APPLN. INFO: US 1999-261358 19990303; US 1998-201458 19981130

Searcher : Shears 308-4994

AN 2000-412298 [35] WPIDS
CR 2002-351882 [38]
AB WO 200032745 A UPAB: 20021022
NOVELTY - DNA sequences (I) encoding a 30 kiloDalton (kDa) protein (II) from **Ehrlichia canis**, which is immuno-reactive with anti-**E. canis** serum, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) a recombinant protein (II) comprising 1 of 3 defined amino acid sequences ((IIa), (IIb) and (IIc)) given in the specification ((IIa), (IIb) and (IIc) are 278, 283 and 280 amino acids in length respectively);

(2) a vector (III) comprising (I);

(3) a host cell (IV) comprising the nucleic acid sequences (Ia), (Ib) and/or (Ic) (3 defined nucleotide sequences given in the specification ((Ia), (Ib) and (Ic) are 1607, 849 and 840 nucleotides in length respectively));

(4) a method (V) of producing (II), comprising:

(a) obtaining a vector (III) comprising an expression region encoding the amino acid sequences (IIa), (IIb) and/or (IIc), operatively linked to a promoter;

(b) transfecting the vector into a cell; and

(c) culturing the cell under conditions suitable for expression of the expression region;

(5) an antibody (VI) immunoreactive with (IIa), (IIb) and/or (IIc); and

(6) a method (VII) of inhibiting **E. canis** infection in a subject comprising:

(a) identifying a subject suspected of being exposed to or infected with **E. canis**; and

(b) administering **28-kDa** antigens from **E. canis**.

ACTIVITY - Antibacterial.

MECHANISM OF ACTION - Vaccine.

Recombinant **E. canis** ECa28-1 fusion protein was subjected to SDS (sodium dodecyl sulfate)-polyacrylamide gel electrophoresis (SDS-PAGE) on 4-15% TrisHCl gradient gels and transferred to pure nitrocellulose using a semi-dry transfer cell. The membrane was incubated with convalescent phase antisera from an **E. canis**-infected **dog** diluted 1:5000 for 1 hour, washed, and then incubated with an anti-**canine** immunoglobulin (Ig)-G alkaline phosphatase-conjugated affinity-purified secondary antibody at 1:1000 for 1 hour. Bound antibody was visualized with 5-bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium (BCIP/NBT) substrate.

USE - (I) may be used for the recombinant production of (II) to obtain antigens that may be used to vaccinate a subject (especially a **dog**) against **E. canis** (claimed, i.e. method (VII)) infections such as **canine ehrlichiosis** (**canine** tropical pancytopenia), which is a tick-borne rickettsial disease of **dogs**. **E. canis** is a small gram-negative, obligate intracellular bacterium that exhibits tropism for mononuclear phagocytes and is transmitted by the brown **dog** tick Rhipicephalus sanguineus. The chronic phase of the illness is characterized by thrombocytopenia, hyperglobulinemia, anorexia, emaciation and hemorrhage (epitaxis) and may result in death however most patients

recover but become carriers of the disease for several years.
Dwg.0/8

L9 ANSWER 17 OF 31 MEDLINE DUPLICATE 6
ACCESSION NUMBER: 2001013459 MEDLINE
DOCUMENT NUMBER: 20432107 PubMed ID: 10974556
TITLE: A conserved, transcriptionally active **p28** multigene locus of **Ehrlichia canis**

AUTHOR: McBride J W; Yu X J; Walker D H
CORPORATE SOURCE: Department of Pathology and WHO Collaborating Center for Tropical Diseases, University of Texas Medical Branch, Galveston, TX 77555-0609, USA.
CONTRACT NUMBER: AI31431 (NIAID)
SOURCE: GENE, (2000 Aug 22) 254 (1-2) 245-52.
Journal code: 7706761. ISSN: 0378-1119.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AF168788; GENBANK-AF168789
ENTRY MONTH: 200010
ENTRY DATE: Entered STN: 20010322
Last Updated on STN: 20010322
Entered Medline: 20001030

AB Antigenic diversity of Ehrlichia chaffeensis and **Ehrlichia canis** may involve independent or differential expression of the **P28** outer membrane proteins genes, enabling persistent infections of the natural hosts. In this study, we analyzed the transcriptional activity of a five gene locus in **E. canis** encoding homologous, but non-identical, **p28** genes. The **p28** multigene locus contained three previously identified complete gene sequences and one partial gene sequence. A new **p28** gene was identified and sequenced, and the complete sequence of a second partial **p28** gene was determined. The new **p28** gene joined two previously separate loci, forming the single **p28** multigene locus. The amino acid homology of the **E. canis** **P28** proteins ranged from 51 to 74%. The nucleic acid sequence of regions compared within the locus spanning four **p28** genes from two geographically distinct **E. canis** isolates was completely conserved. Analysis of the five **p28** genes demonstrated that all were transcriptionally active in in-vitro cultures of **E. canis** incubated at the vertebrate host (37 degrees C) and ambient tick temperatures (27 degrees C). Polycistronic copies of multiple **p28** genes were not detected by RT-PCR, but monocistronic **p28** mRNA transcripts were detected by Northern blotting from **E. canis** infected DH82 cells, indicating that the genes are transcribed as monocistronic messages.

L9 ANSWER 18 OF 31 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2001:3781 BIOSIS
DOCUMENT NUMBER: PREV200100003781
TITLE: Multiple **Ehrlichia canis** **p28** genes are transcriptionally active as monocistronic messages.

AUTHOR(S): McBride, J. W. (1); Yu, X.-J. (1); Walker, D. H. (1)
CORPORATE SOURCE: (1) Department of Pathology, University of Texas Medical Branch, Galveston, TX USA
SOURCE: American Journal of Tropical Medicine and Hygiene, (March, 2000) Vol. 62, No. 3 Supplement, pp. 188. print.
Meeting Info.: 49th Annual Meeting of the American Society of Tropical Medicine and Hygiene Houston, Texas, USA October 29-November 02, 2000 American Society of Tropical Medicine and Hygiene
. ISSN: 0002-9637.
DOCUMENT TYPE: Conference
LANGUAGE: English
SUMMARY LANGUAGE: English

L9 ANSWER 19 OF 31 MEDLINE DUPLICATE 7
ACCESSION NUMBER: 2000033623 MEDLINE
DOCUMENT NUMBER: 20033623 PubMed ID: 10565902
TITLE: Western and dot blotting analyses of Ehrlichia chaffeensis indirect fluorescent-antibody assay-positive and -negative human sera by using native and recombinant E. chaffeensis and **E**
. **canis** antigens.
AUTHOR: Unver A; Rikihisa Y; Ohashi N; Cullman L C; Buller R; Storch G A
CORPORATE SOURCE: Department of Veterinary Biosciences, College of Veterinary Medicine, The Ohio State University, Columbus, Ohio 43210-1093, USA.
CONTRACT NUMBER: AI40934 (NIAID)
SOURCE: JOURNAL OF CLINICAL MICROBIOLOGY, (1999 Dec) 37 (12) 3888-95.
Journal code: 7505564. ISSN: 0095-1137.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200001
ENTRY DATE: Entered STN: 20000124
Last Updated on STN: 20000124
Entered Medline: 20000107

AB Human monocytic **ehrlichiosis** is an emerging infectious disease caused by Ehrlichia chaffeensis, a gram-negative obligatory intracellular bacterium closely related to **E**.
canis. The immunoreactive recombinant fusion proteins rP28 and rP30 have become available after cloning and expressing of the 28- and 30-kDa major outer membrane protein genes of E. chaffeensis and **E**. **canis**, respectively. Western immunoblotting was performed to analyze the antibody responses of the 37 E. chaffeensis indirect fluorescent-antibody assay (IFA)-positive and 20 IFA-negative serum specimens with purified whole organisms, rP28, and rP30. All IFA-negative sera were negative with purified whole organisms, rP28, or rP30 by Western immunoblot analysis (100% relative diagnostic specificity). Of 37 IFA-positive sera, 34 sera reacted with any native proteins of E. chaffeensis ranging from 44 to 110 kDa, and 30 sera reacted with 44- to 110-kDa native **E**. **canis** antigens. The
28-kDa E. chaffeensis and 30-kDa **E**.
canis native proteins were recognized by 25 IFA-positive

sera. Fifteen IFA-positive sera reacted with rP28 by Western blot analysis, whereas 34 IFA-positive sera reacted with rP30 (92% relative diagnostic specificity), indicating that rP30 is more sensitive than rP28 for detecting the antibodies in IFA-positive sera. These 34 IFA-positive sera were positive by the dot blot assay with rP30, distinguishing them from IFA-negative sera. Except for three rP30-negative but IFA-positive specimens that instead showed an E. ewingii infection-like profile by Western immunoblotting, the results of Western and dot blot assays with rP30 matched 100% with the IFA test results. Densitometric analysis of dot blot reactions showed a positive correlation between the dot density and the IFA titer. These results suggest that rP30 antigen would provide a simple, consistent, and rapid serodiagnosis for human monocytic **ehrlichiosis**.

L9 ANSWER 20 OF 31 CABA COPYRIGHT 2003 CABI
ACCESSION NUMBER: 1999:143644 CABA
DOCUMENT NUMBER: 990506067
TITLE: Comparison of Ehrlichia chaffeensis recombinant proteins for serologic diagnosis of human monocytotropic **ehrlichiosis**
AUTHOR: Yu XueJie; Crocquet-Valdes, P. A.; Cullman, L. C.; Popov, V. L.; Walker, D. H.; Yu, X. J.
CORPORATE SOURCE: Department of Pathology, 301 University Blvd., University of Texas Medical Branch, Galveston, TX 77555-0609, USA.
SOURCE: Journal of Clinical Microbiology, (1999) Vol. 37, No. 8, pp. 2568-2575. 38 ref.
ISSN: 0095-1137
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Protein immunoblotting was used to evaluate the reaction of the antibodies in patients' sera with the recombinant E. chaffeensis 120- and **28-kDa** proteins as well as the 106- and the 37-kDa proteins. The cloning of the genes encoding the latter 2 proteins is described. Immunoelectron microscopy demonstrated that the 106-kDa protein is located at the surfaces of ehrlichiae and on the intramorular fibrillar structures associated with E. chaffeensis. The 37-kDa protein is homologous to the iron-binding protein of Gram-negative bacteria. 42 serum samples from patients who were suspected to have human monocytotropic **ehrlichiosis** were tested by immunofluorescence (IFA) using E. chaffeensis antigen and by protein immunoblotting using recombinant E. chaffeensis proteins expressed in Escherichia coli. 32 serum samples contained IFA antibodies at a titre of 1:64 or greater. The correlation of IFA and recombinant protein immunoblotting was 100% for the 120-kDa protein, 41% for the **28-kDa** protein, 9.4% for the 106-kDa protein, and 0% for the 37-kDa protein. None of the recombinant antigens yielded false-positive results. All the sera reactive with the recombinant 28- or the 106-kDa proteins also reacted with the recombinant 120-kDa protein. The EMBL\GenBank\DDBJ accession number for the nucleotide sequences of the 106- and 37-kDa protein genes is AF117273.

L9 ANSWER 21 OF 31 MEDLINE DUPLICATE 8
ACCESSION NUMBER: 1999242757 MEDLINE
DOCUMENT NUMBER: 99242757 PubMed ID: 10225842
TITLE: Molecular cloning of the gene for a conserved major

immunoreactive **28-kilodalton** protein of **Ehrlichia canis**: a potential serodiagnostic antigen.
AUTHOR: McBride J W; Yu X j; Walker D H
CORPORATE SOURCE: Department of Pathology and WHO Collaborating Center for Tropical Diseases, University of Texas Medical Branch, Galveston, Texas 77555-0609, USA.
SOURCE: CLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY, (1999 May) 6 (3) 392-9.
Journal code: 9421292. ISSN: 1071-412X.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AF082744; GENBANK-AF082745; GENBANK-AF082746; GENBANK-AF082747; GENBANK-AF082748; GENBANK-AF082749; GENBANK-AF082750
ENTRY MONTH: 199909
ENTRY DATE: Entered STN: 19990925
Last Updated on STN: 19990925
Entered Medline: 19990916

AB A gene encoding a **28-kDa** protein of **Ehrlichia canis** was cloned, sequenced, and expressed, and a comparative molecular analysis with homologous genes of **E. canis**, Cowdria ruminantium, and Ehrlichia chaffeensis was performed. The complete gene has an 834-bp open reading frame encoding a protein of 278 amino acids with a predicted molecular mass of 30.5 kDa. An N-terminal signal sequence was identified, suggesting that the protein undergoes posttranslational modification to a mature 27.7-kDa protein (**P28**). The **E. canis p28** gene has significant nucleic acid and amino acid sequence homologies with the E. chaffeensis outer membrane protein-1 (omp-1) gene family, with the Cowdria ruminantium map-1 gene, and with other **E. canis 28-kDa**-protein genes. Southern blotting revealed the presence of at least two additional homologous **p28** gene copies in the **E. canis** genome, confirming that **p28** is a member of a polymorphic multiple-gene family. Amino acid sequence analysis revealed that **E. canis P28** has four variable regions, and it shares similar surface-exposed regions, antigenicity, and T-cell motifs with E. chaffeensis **P28**. The **p28** genes from seven different **E. canis** isolates were identical, indicating that the gene for this major immunoreactive protein is highly conserved. In addition, reactivity of sera from clinical cases of **canine ehrlichiosis** with the recombinant **P28** demonstrated that the recombinant protein may be a reliable serodiagnostic antigen.

L9 ANSWER 22 OF 31 MEDLINE
ACCESSION NUMBER: 2000411517 MEDLINE
DOCUMENT NUMBER: 20391235 PubMed ID: 10425222
TITLE: Variability in the **28-kDa** surface antigen protein multigene locus of isolates of the emerging disease agent Ehrlichia chaffeensis suggests that it plays a role in immune evasion.
COMMENT: Erratum in: Mol Cell Biol Res Commun 2000 Jan;3(1):66
AUTHOR: Reddy G R; Streck C P

CORPORATE SOURCE: Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan 66506, USA.. rganta@vet.ksu.edu
SOURCE: MOLECULAR CELL BIOLOGY RESEARCH COMMUNICATIONS, (1999 Jun) 1 (3) 167-75.
Journal code: 100889076. ISSN: 1522-4724.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AF151712; GENBANK-AF151713; GENBANK-AF151714; GENBANK-AF151715
ENTRY MONTH: 200008
ENTRY DATE: Entered STN: 20000907
Last Updated on STN: 20001005
Entered Medline: 20000828

AB Infections caused by rickettsial pathogens persist in vertebrate hosts for long periods of time, despite the active host immune response. We recently described the multigene locus encoding **28 kDa** surface antigen proteins (**28 kDa** SAPs) for two closely related rickettsials, Ehrlichia chaffeensis and **Ehrlichia canis** (Reddy, G. R., et al. (1998) Biochem. Biophys. Res. Commun. 247, 636-643), that share extensive structural homology to antigenic variant surface protein genes of Neisseria and Borrelia species. In this study, we describe motifs for recombinase binding sites and a high frequency of repeat elements in the cloned **28 kDa** SAP genes. The locus for two newly established E. chaffeensis isolates also was characterized, and immunological specificity of the **28 kDa** SAPs was investigated. The study identified variant forms of the **28 kDa** SAPs and extensive restriction fragment length polymorphisms among isolates. The molecular data suggest that the locus is influenced by short-term evolutionary changes such as genetic recombinations leading to the generation of antigenic variants. Antigenic variation could contribute to the mechanism of immune evasion and the emergence of new diseases.

L9 ANSWER 23 OF 31 MEDLINE DUPLICATE 9
ACCESSION NUMBER: 1998371112 MEDLINE
DOCUMENT NUMBER: 98371112 PubMed ID: 9705412
TITLE: Cloning and characterization of multigenes encoding the immunodominant 30-kilodalton major outer membrane proteins of **Ehrlichia canis** and application of the recombinant protein for serodiagnosis.
AUTHOR: Ohashi N; Unver A; Zhi N; Rikihisa Y
CORPORATE SOURCE: Department of Veterinary Biosciences, College of Veterinary Medicine, The Ohio State University, Columbus, Ohio 43210-1093, USA.
CONTRACT NUMBER: RO1 AI33123 (NIAID)
SOURCE: JOURNAL OF CLINICAL MICROBIOLOGY, (1998 Sep) 36 (9) 2671-80.
Journal code: 7505564. ISSN: 0095-1137.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals

OTHER SOURCE: GENBANK-AF078553; GENBANK-AF078554; GENBANK-AF078555
ENTRY MONTH: 199809
ENTRY DATE: Entered STN: 19980925
Last Updated on STN: 20000303
Entered Medline: 19980916

AB A 30-kDa major outer membrane protein of **Ehrlichia canis**, the agent of **canine ehrlichiosis**, is the major antigen recognized by both naturally and experimentally infected **dog** sera. The protein cross-reacts with a serum against a recombinant **28-kDa** protein (rP28), one of the outer membrane proteins of a gene (omp-1) family of Ehrlichia chaffeensis. Two DNA fragments of **E. canis** were amplified by PCR with two primer pairs based on the sequences of E. chaffeensis omp-1 genes, cloned, and sequenced. Each fragment contained a partial 30-kDa protein gene of **E. canis**. Genomic Southern blot analysis with the partial gene probes revealed the presence of multiple copies of these genes in the **E. canis** genome. Three copies of the entire gene (p30, p30-1, and p30a) were cloned and sequenced from the **E. canis** genomic DNA. The open reading frames of the two copies (p30 and p30-1) were tandemly arranged with an intergenic space. The three copies were similar but not identical and contained a semivariable region and three hypervariable regions in the protein molecules. The following genes homologous to three **E. canis** 30-kDa protein genes and the E. chaffeensis omp-1 family were identified in the closely related rickettsiae: wsp from Wolbachia sp. , p44 from the agent of human granulocytic **ehrlichiosis**, msp-2 and msp-4 from Anaplasma marginale, and map-1 from Cowdria ruminantium. Phylogenetic analysis among the three **E. canis** 30-kDa proteins and the major surface proteins of the rickettsiae revealed that these proteins are divided into four clusters and the two **E. canis** 30-kDa proteins are closely related but that the third 30-kDa protein is not. The p30 gene was expressed as a fusion protein, and the antibody to the recombinant protein (rP30) was raised in a mouse. The antibody reacted with rP30 and a 30-kDa protein of purified **E. canis**. Twenty-nine indirect fluorescent antibody (IFA)-positive **dog** plasma specimens strongly recognized the rP30 of **E. canis** . To evaluate whether the rP30 is a suitable antigen for serodiagnosis of **canine ehrlichiosis**, the immunoreactions between rP30 and the whole purified **E. canis** antigen were compared in the dot immunoblot assay. Dot reactions of both antigens with IFA-positive **dog** plasma specimens were clearly distinguishable by the naked eye from those with IFA-negative plasma specimens. By densitometry with a total of 42 IFA-positive and -negative plasma specimens, both antigens produced results similar in sensitivity and specificity. These findings suggest that the rP30 antigen provides a simple, consistent, and rapid serodiagnosis for **canine ehrlichiosis**. Cloning of multigenes encoding the 30-kDa major outer membrane proteins of **E. canis** will greatly facilitate understanding pathogenesis and immunologic study of **canine** ehrlichosis and provide a useful tool for phylogenetic analysis.

L9 ANSWER 24 OF 31 MEDLINE DUPLICATE 10
ACCESSION NUMBER: 1998321180 MEDLINE

DOCUMENT NUMBER: 98321180 PubMed ID: 9647746
TITLE: Molecular characterization of a **28 kDa** surface antigen gene family of the tribe Ehrlichiae.
AUTHOR: Reddy G R; Sulsona C R; Barbet A F; Mahan S M; Burridge M J; Alleman A R
CORPORATE SOURCE: Department of Pathobiology, College of Veterinary Medicine, University of Florida, Gainesville 32610, USA.
SOURCE: BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, (1998 Jun 29) 247 (3) 636-43.
Journal code: 0372516. ISSN: 0006-291X.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AF062761; GENBANK-AF062762
ENTRY MONTH: 199807
ENTRY DATE: Entered STN: 19980817
Last Updated on STN: 19980817
Entered Medline: 19980731

AB Antisera against different Ehrlichiae recognize an immunodominant, cross-reacting approximately **28 kDa** surface antigen defined as the MAP1 in Cowdria ruminantium. These antigens are considered valuable in developing serodiagnostic tests and recombinant vaccines for Ehrlichiae infections. To evaluate the relationship in three closely related Ehrlichiae, Ehrlichia chaffeensis, **Ehrlichia canis**, and C. ruminantium, the structure of the **28 kDa** antigen genes was analyzed. We describe the cloning and characterization of DNA encoding genes homologous to MAP1 from E. chaffeensis and **E. canis**. The cloned segment of E. chaffeensis contains one expressed and four transcriptionally silent tandemly arranged, nonidentical genes; the **E. canis** locus consists of two nonidentical genes. Comparative analysis of these genes revealed the presence of four conserved regions separated by three highly variable regions. B-cell epitope analysis identified three major cross-reacting epitopes that map to the variable regions. Location of the epitopes at the variable regions and the presence of multigene family with only one expressed copy suggest a mechanism of immune evasion in these Ehrlichiae.

L9 ANSWER 25 OF 31 MEDLINE DUPLICATE 11
ACCESSION NUMBER: 1998084465 MEDLINE
DOCUMENT NUMBER: 98084465 PubMed ID: 9423849
TITLE: Immunodominant major outer membrane proteins of Ehrlichia chaffeensis are encoded by a polymorphic multigene family.
AUTHOR: Ohashi N; Zhi N; Zhang Y; Rikihisa Y
CORPORATE SOURCE: Department of Veterinary Biosciences, College of Veterinary Medicine, The Ohio State University, Columbus 43210-1093, USA.
CONTRACT NUMBER: RO1 AI33123 (NIAID)
SOURCE: INFECTION AND IMMUNITY, (1998 Jan) 66 (1) 132-9.
Journal code: 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English

FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AF021338; GENBANK-U72291
ENTRY MONTH: 199801
ENTRY DATE: Entered STN: 19980206
Last Updated on STN: 20000303
Entered Medline: 19980127

AB Several immunodominant major proteins ranging from 23 to 30 kDa were identified in the outer membrane fractions of Ehrlichia chaffeensis and **Ehrlichia canis**. The N-terminal amino acid sequence of a **28-kDa** protein of E. chaffeensis (one of the major proteins) was determined. The gene (**p28**), almost full length, encoding the **28-kDa** protein was cloned by PCR with primers designed based on the N-terminal sequence of the E. chaffeensis **28-kDa** protein and the consensus sequence between the C termini of the Cowdria ruminantium MAP-1 and Anaplasma marginale MSP-4 proteins. The **p28** gene was overexpressed, and antibody to the recombinant protein was raised in a rabbit. The antibody and serum from a patient infected with E. chaffeensis reacted with the recombinant protein, three proteins (29, 28, and 25 kDa) of E. chaffeensis, and a 30-kDa protein of **E. canis**. Immunoelectron microscopy with the rabbit antibody revealed that the antigenic epitope of the **28-kDa** protein was exposed on the surface of E. chaffeensis. Southern blot analysis with a 32P-labeled **p28** gene probe revealed multiple copies of genes homologous to **p28** in the E. chaffeensis genome. Six copies of the **p28** gene were cloned and sequenced from the genomic DNA by using the same probe. The open reading frames of these gene copies were tandemly arranged with intergenic spaces. They were nonidentical genes and contained a semivariable region and three hypervariable regions in the predicted protein molecules. One of the gene copies encoded a protein with an internal amino acid sequence identical to the chemically determined N-terminal amino acid sequence of a 23-kDa protein of E. chaffeensis. Immunization with the recombinant **P28** protein protected mice from infection with E. chaffeensis. These findings suggest that the 30-kDa-range proteins of E. chaffeensis represent a family of antigenically related homologous proteins encoded by a single gene family.

L9 ANSWER 26 OF 31 MEDLINE DUPLICATE 12
ACCESSION NUMBER: 1998043955 MEDLINE
DOCUMENT NUMBER: 98043955 PubMed ID: 9384299
TITLE: Western immunoblotting analysis of the antibody responses of patients with human monocytotropic **ehrlichiosis** to different strains of Ehrlichia chaffeensis and **Ehrlichia canis**.
AUTHOR: Chen S M; Cullman L C; Walker D H
CORPORATE SOURCE: Department of Pathology, University of Texas Medical Branch, Galveston 77555-0609, USA.
CONTRACT NUMBER: AI31431 (NIAID)
SOURCE: CLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY, (1997 Nov) 4 (6) 731-5.
Journal code: 9421292. ISSN: 1071-412X.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English

Searcher : Shears 308-4994

FILE SEGMENT: Priority Journals
ENTRY MONTH: 199801
ENTRY DATE: Entered STN: 19980122
Last Updated on STN: 19980122
Entered Medline: 19980107

AB In order to evaluate the relative sensitivity of the detection of antibodies against various antigenic proteins of Ehrlichia chaffeensis for the diagnosis of the emerging infectious disease human monocytotropic **ehrlichiosis**, Western immunoblotting was performed with 27 serum samples from convalescent patients with antibodies, as demonstrated by indirect immunofluorescence assay. Among 22 patients with antibodies reactive with the 120-kDa protein, 15 showed reactivity with the 29/**28-kDa** protein(s) and the proteins in the 44- to 88-kDa range. Two of the serum samples with this pattern reacted with the 29/**28-kDa** protein(s) of only the 91HE17 strain, and one sample reacted with only that of the Arkansas strain, indicating that the antibodies were stimulated by strain-specific epitopes. Overall, antibodies to the 29/**28-kDa** protein(s) were detected in only 16 patients' sera, suggesting that this protein is less sensitive than the 120-kDa protein. Two of 12 serum samples from healthy blood donors had antibodies reactive with the 120-kDa protein; one of these samples reacted also with the 29/**28-kDa** protein(s) of **Ehrlichia canis**, suggesting that unrecognized ehrlichial infection might have occurred, including human infection with **E. canis**. A high correlation between reactivity with the 120-kDa protein by Western immunoblotting and the recombinant 120-kDa protein by dot blot supports the potential usefulness of this recombinant antigen in diagnostic serology.

L9 ANSWER 27 OF 31 MEDLINE DUPLICATE 13
ACCESSION NUMBER: 96208049 MEDLINE
DOCUMENT NUMBER: 96208049 PubMed ID: 8615456
TITLE: Analysis and ultrastructural localization of Ehrlichia chaffeensis proteins with monoclonal antibodies.
AUTHOR: Chen S M; Popov V L; Feng H M; Walker D H
CORPORATE SOURCE: Department of Pathology, University of Texas Medical Branch, Galveston, USA.
CONTRACT NUMBER: AI-314131 (NIAID)
SOURCE: AMERICAN JOURNAL OF TROPICAL MEDICINE AND HYGIENE, (1996 Apr) 54 (4) 405-12.
Journal code: 0370507. ISSN: 0002-9637.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 199606
ENTRY DATE: Entered STN: 19960613
Last Updated on STN: 19960613
Entered Medline: 19960606

AB Ehrlichia chaffeensis, an obligately intracellular bacterium with tropism for monocytes, is the etiologic agent of human monocytic **ehrlichiosis**. To determine the nature and ultrastructural location of E. chaffeensis antigens, monoclonal antibodies (MAbs) to E. chaffeensis were developed. The MAbs were used for immunofluorescence and Western immunoblotting analysis of the

antigens of density gradient-purified ehrlichiae. Monoclonal antibody 6A1 recognized an epitope of a 30-kD protein. This antibody reacted with a strain-specific epitope of E. chaffeensis, Arkansas strain, and did not cross-react with any other ehrlichia tested. Monoclonal antibodies 3C7 and 7C1-B recognized Arkansas strain proteins of 30 and 29 kD and reacted with E. chaffeensis (strain 91HE17) proteins of 31 and 29 kD and an **E. canis** protein of 30 kD. Lack of reactivity of these two MAbs with E. sennetsu and E. risticii suggests that the epitope is group-specific. Monoclonal antibody 5D11 recognized a 58-kD protein of both strains of E. chaffeensis as well as **E. canis**, apparently a group-specific, conformation-independent epitope. Monoclonal antibody 7C1-C reacted with 58- and 88-kD proteins of both the Arkansas and 91HE17 strains. Trypsin treatment destroyed the reactivity of E. chaffeensis antigens with all the MAbs when tested by Western immunoblotting, indicating that these antigens are proteins with trypsin-sensitive epitopes. Immunoelectron microscopy of negatively stained intact E. chaffeensis organisms showed that the 30- and 29-kD proteins are present on the surface of the ehrlichial cell wall along with the previously localized **28-kD** protein.

L9 ANSWER 28 OF 31 CABA COPYRIGHT 2003 CABI
ACCESSION NUMBER: 96:18385 CABA
DOCUMENT NUMBER: 960500451
TITLE: Serologic diagnosis of human monocytic **ehrlichiosis** by immunoblot analysis
AUTHOR: Brouqui, P.; Lecam, C.; Olson, J.; Raoult, D.
CORPORATE SOURCE: Unite des Rickettsies, Faculte de Medecine, 13385 Marseilles cedex 5, France.
SOURCE: Clinical and Diagnostic Laboratory Immunology, (1995) Vol. 1, No. 6, pp. 645-649. 28 ref. ISSN: 1071-412X
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Human monocytic **ehrlichiosis** is caused by Ehrlichia chaffeensis. Despite its lack of specificity in discriminating among infections by closely related Ehrlichia spp., immunofluorescence assay (IFA) is the most frequently used serological diagnostic method. To improve the specificity of the serological diagnosis, the antigenic profile of **E. canis** and E. chaffeensis antigen was compared with homologous and heterologous sera, searching for the specificity of the presence of low-molecular-weight proteins. Western immunoblot analysis of IFA-positive human sera revealed 27- and 29-kDa proteins which are not found in **E. canis** IFA-positive sera from **dogs**. IFA-positive sera from **dogs** revealed a low-molecular-weight group of proteins (20-**28 kDa**) which were not found in human E. chaffeensis-positive sera except for a weak band at 22 kDa. The presence of antibodies directed against the 27- and 29-kDa proteins on Western blots is specific for E. chaffeensis infection, and it is suggested that the Western blot might complete IFA in cases with low positive predictive value.

L9 ANSWER 29 OF 31 MEDLINE DUPLICATE 14
ACCESSION NUMBER: 96050885 MEDLINE
DOCUMENT NUMBER: 96050885 PubMed ID: 8556515
TITLE: Serologic diagnosis of human monocytic

ehrlichiosis by immunoblot analysis.
AUTHOR: Brouqui P; Lecam C; Olson J; Raoult D
CORPORATE SOURCE: Unite des Rickettsies, Faculte de Medecine, Marseilles, France.
SOURCE: CLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY, (1994 Nov) 1 (6) 645-9.
Journal code: 9421292. ISSN: 1071-412X.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199602
ENTRY DATE: Entered STN: 19960312
Last Updated on STN: 19980206
Entered Medline: 19960223

AB Human monocytic **ehrlichiosis** is caused by Ehrlichia chaffeensis, an intracellular bacterium probably transmitted by the tick Amblyomma americanum in the United States. Despite its lack of specificity in discriminating among infections by closely related Ehrlichia spp., immunofluorescence assay (IFA) is the most frequently used serological diagnostic method. To improve the specificity of the serological diagnosis, we compared antigenic profile of **E. canis** and E. chaffeensis antigen with homologous and heterologous sera, searching for the specificity of the presence of low-molecular-weight proteins. Western immunoblot analysis of IFA-positive human sera revealed 27- and 29-kDa proteins which are not found in **E. canis** IFA-positive sera from **dogs**. IFA-positive sera from **dogs** revealed a low-molecular-weight group of proteins (20 to **28 kDa**) which were not found in human E. chaffeensis-positive sera except for a weak band at 22 kDa. The presence o antibodies directed against the 27- and 29-kDa proteins on Western blots is specific for E. chaffeensis infection, and we suggest that the Western blot might complete IFA in cases with low positive predictive value.

L9 ANSWER 30 OF 31 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1994:330641 BIOSIS
DOCUMENT NUMBER: PREV199497343641
TITLE: Serologic diagnosis of human monocytic **ehrlichiosis** using Western immunoblots: 22-**28 kDa** immunogenic proteins are species.
AUTHOR(S): Brouqui, P. (1); Le Cam, C.; Olson, J.; Raoult, D.
CORPORATE SOURCE: (1) Unite Rickettsies, Marseille France
SOURCE: Abstracts of the General Meeting of the American Society for Microbiology, (1994) Vol. 94, No. 0, pp. 101.
Meeting Info.: 94th General Meeting of the American Society for Microbiology Las Vegas, Nevada, USA May 23-27, 1994
ISSN: 1060-2011.
DOCUMENT TYPE: Conference
LANGUAGE: English

L9 ANSWER 31 OF 31 CONFSCI COPYRIGHT 2003 CSA
ACCESSION NUMBER: 2000:70499 CONFSCI
DOCUMENT NUMBER: 00-067370

TITLE: Multiple **Ehrlichia canis** **P28** genes are transcriptionally active as monocistronic messages
AUTHOR: McBride, J.W.; Yu, X.-J.; Walker, D.H.
CORPORATE SOURCE: Dep. Pathol., Univ. Texas Med. Branch, Galveston, TX, USA
SOURCE: American Society of Tropical Medicine and Hygiene, 3088 Briarcliff Rd., Ste. 11A, Atlanta, GA 30329, USA

Meeting Info.: 000 5172: ASTMH 49th Annual Meeting (0005172). Houston, Texas (USA). 29 Oct-2 Nov 2000. American Society of Tropical Medicine and Hygiene.
DOCUMENT TYPE: Conference
FILE SEGMENT: DCCP
LANGUAGE: English

(FILE 'MEDLINE' ENTERED AT 10:51:28 ON 09 JUL 2003)
L10 10 SEA FILE=MEDLINE ABB=ON PLU=ON "EHRLICHIA CANIS"/CT
L11 886 SEA FILE=MEDLINE ABB=ON PLU=ON EHRLICHIOSIS/CT
L12 9 SEA FILE=MEDLINE ABB=ON PLU=ON L10 AND L11

L12 ANSWER 1 OF 9 MEDLINE
AN 2003234255 MEDLINE
TI Serologic prevalence of Dirofilaria immitis, Ehrlichia canis, and Borrelia burgdorferi infections in Brazil.
AU Labarthe Norma; de Campos Pereira Marcelo; Barbarini Oclydes; McKee William; Coimbra Carlos Alberto; Hoskins Johnny
SO Vet Ther, (2003 Spring) 4 (1) 67-75.
Journal code: 100936368. ISSN: 1528-3593.
AB Dogs infected with Dirofilaria immitis, Ehrlichia canis, or Borrelia burgdorferi may show nonspecific clinical signs or may be asymptomatic. In Brazil, E. canis and D. immitis infections are frequently diagnosed based on the presence of classical signs; however, serologic tests are seldom performed to confirm the presence of infection. To estimate the seroprevalence of these three canine diseases in Brazil, 2,553 dogs presented at veterinary practices for various tests, routine treatments, or examinations were evaluated by an in-office commercial ELISA test kit (SNAP 3Dx, IDEXX Laboratories). Each dog was examined by the veterinarian, and a whole-blood sample was collected and immediately tested for the simultaneous detection of B. burgdorferi and E. canis antibodies and D. immitis antigen. D. immitis infection was detected in 51 dogs (2.0%) and E. canis antibodies were present in 505 dogs 19.8%). Only one dog tested positive for B. burgdorferi antibodies.

L12 ANSWER 2 OF 9 MEDLINE
AN 2003139725 MEDLINE
TI Cloning and characterization of an Ehrlichia canis gene encoding a protein localized to the morula membrane.
AU Teng Ching-Hao; Palaniappan Raghavan U M; Chang Yung-Fu
SO INFECTION AND IMMUNITY, (2003 Apr) 71 (4) 2218-25.
Journal code: 0246127. ISSN: 0019-9567.
AB A gene encoding a 23.5-kDa ehrlichial morula membrane protein designated MmpA was cloned by screening an Ehrlichia canis expression library with convalescent dog sera, which resulted in three positive clones. Sequence analysis of the insert DNAs from all three clones indicated an open reading frame with a size of 666 bp that encodes MmpA. The structural analysis of MmpA indicated

that it is a transmembrane protein with extreme hydrophobicity. Southern blot analysis of the HindIII-digested chromosomal DNA demonstrated the presence of a single copy of the mmpA gene in E. canis and Ehrlichia chaffeensis but not in the human granulocytic ehrlichiosis agent. The mmpA gene was amplified, cloned, and expressed as a fusion protein. Polyclonal antibodies to the recombinant protein (rMmpA) were raised in rabbits. Western blot analysis of E. canis and E. chaffeensis lysates with the anti-rMmpA serum resulted in the presence of an MmpA band only in E. canis, not in E. chaffeenesis. Sera from dogs which were either naturally or experimentally infected with E. canis recognized the recombinant protein. Double immunofluorescence confocal microscopy studies demonstrated that MmpA was localized mainly on the morula membrane of E. canis. Since the morula membrane is the interface between the ehrlichial growing environment and the host cytoplasm, MmpA may play a role in bacterium-host cell interactions.

L12 ANSWER 3 OF 9 MEDLINE
AN 2003129538 MEDLINE
TI [Serologic evidence for human Ehrlichiosis in Chile]. Ehrlichiosis humana en Chile, evidencia serologica.
AU Lopez Javier; Rivera Marisol; Concha Juan Carlos; Gatica Silvana; Loeffeholz Mike; Barriga Omar
SO REVISTA MEDICA DE CHILE, (2003 Jan) 131 (1) 67-70. Journal code: 0404312. ISSN: 0034-9887.
AB BACKGROUND: Ehrlichiosis is a non contagious infectious disease, mainly transmitted by thick bites. In 1998, this infection was detected in dogs, for the first time, in Chile. AIM: To establish if there is human exposure to Ehrlichia sp in Chile. MATERIAL AND METHODS: Blood samples from 17 dogs with ehrlichiosis and 19 humans who had contact with them were studied to determine human exposure to Ehrlichia equi and Ehrlichia chaffeensis in Chile. Samples were analyzed by indirect immunofluorecence and by polymerase chain reaction (PCR). RESULTS: Six dogs had positive titers against both species of ehrlichia; 2 with titers of 1/256; 3 with titers over 1/512 to Ehrlichia equi and titers of 1/256, 1/128 and 1/64 to Ehrlichia chaffeensis respectively, and 1 with titers of 1/256 to Ehrlichia equi and titers of 1/128 to Ehrlichia chaffeensis. Two of the 19 humans, had positive titers against both antigens (1/128). PCR reactions were negative in both human and canine sera. CONCLUSIONS: These results confirm that human exposure to Ehrlichia sp. Epidemiological surveillance for human ehrlichiosis should be implemented in the country.

L12 ANSWER 4 OF 9 MEDLINE
AN 2003072351 MEDLINE
TI Assay of fipronil efficacy to prevent canine monocytic ehrlichiosis in endemic areas.
AU Davoust B; Marie J L; Mercier S; Boni M; Vandeweghe A; Parzy D; Beugnet F
SO VETERINARY PARASITOLOGY, (2003 Feb 28) 112 (1-2) 91-100. Journal code: 7602745. ISSN: 0304-4017.
AB Our objective was to evaluate the efficacy of fipronil for the prevention of Ehrlichia canis transmission to dogs by Rhipicephalus sanguineus in two endemic areas situated in Africa (Dakar and Djibouti). We carried out controlled trials in kennels for 1 year on 248 dogs, mainly police dogs and military working dogs. Eight groups were studied in a multi-centre study. Fifty five fipronil

treated dogs were located in two separated kennels (G3, 37 dogs in Djibouti and G8, 18 dogs in Dakar). G1 (66 dogs) and G2 (60 dogs) were untreated control groups located in Djibouti, whereas G4 (32 dogs), G5 (13 dogs), G6 (18 dogs) and G7 (4 dogs) were the control groups located in Dakar. The epidemiological status of each group is known. G1 and G2 dogs were not kept in kennels, whereas G3, G4, G5, G6, G7, G8 dogs were housed in equivalent kennels. Tick infestation, clinical status and Ehrlichia seroprevalence were assessed during 1 year (duration of the study). Dog treated with fipronil showed neither canine monocytic ehrlichiosis (CME) nor tick infestations. In all groups of untreated control animals, R. sanguineus tick infestations were frequent, particularly in kennels (G5, G6 and G7) as well as morbidity and mortality due to CME. E. canis infection rates were low for fipronil treated animals: 2.7% (1/37) for G3 and 5.5% (1/18) for G8 group. Among control animals, seroprevalence was maximum (100%) in dogs kept in kennels (G5, G6 and G7 groups) and high among native dogs in Djibouti (G1 group): 69.7% (46/66) and in Dakar (G4 group): 50% (16/32). Dogs belonging to expatriate citizens (G2 group) were less likely to be infected: 21.7% (13/60). The comparison of serological results among French army dogs and French citizen dogs that were introduced in Djibouti for an average of 10 months shows a statistically significant ($P<0.001$) difference. Among fipronil treated animals (G3 group), 2 dogs out of 55 seroconverted (3.6%) compared to 13 out of 60 dogs (21.7%) in the control G2 group. The results of our study indicate the preventative efficacy of a fipronil monthly treatment to avoid CME in endemic areas. Epidemiological data concerning animals that live in the same endemic areas are an example of the serious consequences (in terms of mortality and morbidity) that are related to the absence of efficient methods for tick-control. In order to protect dogs that are in transit in endemic areas against tick-transmitted diseases, the use of an adapted acaricide product is recommended.

L12 ANSWER 5 OF 9 MEDLINE
AN 2002707593 MEDLINE
TI Differential serological testing by simultaneous indirect immunofluorescent antibody test in canine leishmaniosis and ehrlichiosis.
AU Guillen Llera J L; Lopez Garcia M L; Martin Reinoso E; De Vivar Gonzalez R
SO VETERINARY PARASITOLOGY, (2002 Nov 11) 109 (3-4) 185-90. Journal code: 7602745. ISSN: 0304-4017.
AB A mixed indirect fluorescence antibody test (IFAT), based on cultured promastigotes Leishmania infantum and formol-inactivated suspension of cells infected with the bacteria Ehrlichia canis, was applied to make a differential diagnosis between canine ehrlichiosis and leishmaniosis. A titre greater than 80 was considered positive for antibodies to E. canis and suggestive of antibodies to L. infantum. Positive sera were titrated subsequently by serial dilutions to confirm antibodies positive to Leishmania and establishing the antibody titre of both pathogens. Fluorescence was absent with negative control sera and background staining was minimal. No serological cross-reactions between positive sera for L. infantum or E. canis were detected. Results obtained by mixed IFAT did not differ when the same serum IFAT standard was compared. The test showed equivalent sensitivity (100%). The specifities were 100% for L. infantum and 98.5% for E. canis. The equivalence in

sensitivity was confirmed by calculating the correlation coefficient between IFAT standards and mixed IFAT (r>or=0.99 for both pathogens). The results of our investigations demonstrated that mixed IFAT is a specific means of establishing serological differential diagnosis of canine leishmaniosis and ehrlichiosis.

L12 ANSWER 6 OF 9 MEDLINE
AN 2002703872 MEDLINE
TI Molecular evidence supporting Ehrlichia canis-like infection in cats.
AU Breitschwerdt Edward B; Abrams-Ogg Anthony C G; Lappin Michael R; Bienzle Dorothee; Hancock Susan I; Cowan Sara M; Clooten Jennifer K; Hegarty Barbara C; Hawkins Eleanor C
SO JOURNAL OF VETERINARY INTERNAL MEDICINE, (2002 Nov-Dec) 16 (6) 642-9.
Journal code: 8708660. ISSN: 0891-6640.
AB Currently, the pathogenic role of Ehrlichia canis in cats has been proposed predominantly on the basis of the serologic evidence of natural infection and the infrequent detection of morulae-like structures within the cytoplasm of leukocytes in cats. The purpose of this report was to provide molecular evidence supporting E. canis-like infection in 3 cats that had clinical manifestations consistent with canine ehrlichiosis but lacked antibodies to E. canis antigens. Serum from all 3 cats contained antinuclear antibodies (ANAs). The predominant disease manifestation was polyarthritis in 1 cat and bone marrow hypoplasia or dysplasia. accompanied by pancytopenia or anemia and thrombocytopenia, in 1 cat each. The alignment of E. canis partial 16S ribosomal DNA (rDNA: 382 nucleotide positions), amplified from EDTA blood samples from each cat, was identical to each other and was identical to a canine isolate of E. canis (GenBank accession number AF373613). In 1 cat, concurrent treatment with corticosteroids may have interfered with the therapeutic effectiveness of doxycycline for the elimination of E. canis-like infection. To further define the spectrum of ehrlichiosis in cats, polymerase chain reaction (PCR) testing may be necessary until serologic testing is thoroughly validated in experimentally or naturally infected cats. In addition, until E. canis has been isolated from cats and several tissue culture isolates are available from disparate geographic regions for detailed comparative genetic study, the molecular evidence presented in this study supporting E. canis-like infection in cats must be interpreted with caution.

L12 ANSWER 7 OF 9 MEDLINE
AN 2002703871 MEDLINE
TI Ehrlichiosis in cats.
AU Legendre Alfred M
SO JOURNAL OF VETERINARY INTERNAL MEDICINE, (2002 Nov-Dec) 16 (6) 641.
Journal code: 8708660. ISSN: 0891-6640.

L12 ANSWER 8 OF 9 MEDLINE
AN 2002697222 MEDLINE
TI Evaluation of cytology in the diagnosis of acute canine monocytic ehrlichiosis (Ehrlichia canis): a comparison between five methods.
AU Mylonakis M E; Koutinas A F; Billinis C; Leontides L S; Kontos V; Papadopoulos O; Rallis T; Fytianou A
SO VETERINARY MICROBIOLOGY, (2003 Feb 2) 91 (2-3) 197-204.
Journal code: 7705469. ISSN: 0378-1135.

AB The purpose of this study was the comparison of the diagnostic sensitivity between buffy coat (BC), peripheral blood (PB), lymph node (LN), bone marrow (BM) and short-term culture (P-D) cytology that has been based on the detection of Ehrlichia canis morulae, in the acute phase of canine monocytic ehrlichiosis (CME). Their cellular localization, total numbers and microscopic differentials were also investigated. The highest sensitivities were achieved after evaluating 1000 oil immersion fields (OIFs) in BC (66%) and an equal number in LN (60.9%) smears, separately or together (74%). The morulae were more often detected into lymphocytes than monocytes. The highest total number of morulae (n=143) were found in P-D smears. Finally, to avoid false positive diagnoses, platelets, lymphocytic azurophilic granules, lymphoglandular bodies and phagocytosed nuclear material should not be confused with the morulae.

L12 ANSWER 9 OF 9 MEDLINE
AN 2002347302 MEDLINE
TI Monitoring C-reactive protein in beagle dogs experimentally inoculated with Ehrlichia canis.
AU Shimada T; Ishida Y; Shimizu M; Nomura M; Kawato K; Iguchi K; Jinbo T
SO VETERINARY RESEARCH COMMUNICATIONS, (2002 Apr) 26 (3) 171-7. Journal code: 8100520. ISSN: 0165-7380.
AB The concentrations of C-reactive proteins (CRP) in the plasma of five beagle dogs experimentally inoculated with Ehrlichia canis increased markedly. The concentrations began to increase between 4 and 16 days and peaked between 15 and 42 days after inoculation of E. canis. The peak concentrations ranged from 217.8 to 788.8 microg/ml (452.6 +/- 228.1 SD). After the peak, the concentrations of CRP decreased rapidly. The PCR product of 16S rRNA of E. canis became detectable in the five dogs between 18 and 27 days after inoculation of E canis. Antibodies to E canis were detected in plasma from the dogs between 5 and 15 days after inoculation of E. canis. The timings of seroconversion and of the start of the increase in CRP were approximately similar and the high concentrations of CRP in the plasma of the dogs tended to become apparent when the PCR product of 16 S rRNA of E. canis became detectable.

(FILE 'HCAPLUS, MEDLINE, BIOSIS, EMBASE, WPIDS, CONFSCI, SCISEARCH, JICST-EPLUS, JAPIO, CABA, AGRICOLA, VETU, VETB' ENTERED AT 10:52:26 ON 09 JUL 2003)

- Author (S)

L13 14938 S "WALKER D"?/AU
L14 11219 S "YU X"?/AU
L15 3384 S "MCBRIDE J"?/AU
L16 57 S L13 AND L14 AND L15
L17 183 S L13 AND (L14 OR L15)
L18 57 S L14 AND L15

L20 53 S (L16 OR L17 OR L18 OR L13 OR L14 OR L15) AND L4
L21 17 DUP REM L20 (36 DUPLICATES REMOVED)

L21 ANSWER 1 OF 17 HCAPLUS COPYRIGHT 2003 ACS DUPLICATE 1
ACCESSION NUMBER: 2003:339170 HCAPLUS
DOCUMENT NUMBER: 138:367457
TITLE: Kinetics of antibody response to

Ehrlichia canis immunoreactive proteins

AUTHOR(S): **McBride, Jere W.**; Corstvet, Richard E.; Gaunt, Steven D.; Boudreaux, Charles; Guedry, Thaya; **Walker, David H.**

CORPORATE SOURCE: Department of Pathology, University of Texas Medical Branch, Galveston, TX, 77555-0609, USA

SOURCE: Infection and Immunity (2003), 71(5), 2516-2524
CODEN: INFIBR; ISSN: 0019-9567

PUBLISHER: American Society for Microbiology

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Immunoreactive proteins of **Ehrlichia canis** and Ehrlichia chaffeensis that have been characterized include a family of **28-kDa** major outer membrane proteins (**p28**) and two large antigenically divergent surface glycoprotein orthologs. We previously demonstrated that recombinant **E. canis p28** and the 140- and 200-kDa glycoproteins gp140 and gp200, resp., react strongly with serum antibodies from suspect canine ehrlichiosis cases that were pos. for **E. canis** by immunofluorescent antibody test and in various phases of acute or chronic infection (2001). The kinetics of the antibody response to these potentially important vaccine and immunodiagnostic candidates is not known. Acute-phase serum antibody responses to whole-cell **E. canis** lysates and recombinant **p28**, gp140, and gp200 were monitored for 6 wk in dogs exptl. infected with **E. canis**. Irresp. of the inoculation route, a T-helper 1-type response was elicited to **E. canis** antigens consisting of IgG2 antibodies exclusively in both acute and convalescent phases in most dogs. Anal. of immunoreactive antigens for peak intensity and relative quantity identified major immunoreactive **E. canis** antigens recognized early in the infection as the 19-, 37-, 75-, and 140-kDa proteins. Later in infection, addnl. major immunoreactive **E. canis** proteins were identified, including the 28-, 47-, and 95-kDa proteins and the recently identified 200-kDa glycoprotein. All dogs had developed antibody against the recombinant gp140, gp200, and **p28** in the convalescent phase. Immunoreactivity and antibody response kinetics suggest that major immunoreactive proteins identified are immunodominant, but early recognition suggests increased dominance by some antigens.

REFERENCE COUNT: 48 THERE ARE 48 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L21 ANSWER 2 OF 17 HCAPLUS COPYRIGHT 2003 ACS DUPLICATE 2

ACCESSION NUMBER: 2002:220752 HCAPLUS

DOCUMENT NUMBER: 136:242995

TITLE: Homologous **28-kDa** immunodominant outer membrane protein genes of **Ehrlichia canis** and uses thereof for dog vaccine preparation to treat related infection

INVENTOR(S): **Walker, David H.**; **Yu, Xue-Jie** ; **Mcbride, Jere W.**

PATENT ASSIGNEE(S): Research Development Foundation, USA

SOURCE: PCT Int. Appl., 106 pp.

CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 4
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002022782	A2	20020321	WO 2001-US28759	20010912
WO 2002022782	A3	20020530		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
US 6392023	B1	20020521	US 2000-660587	20000912
AU 2001090926	A5	20020326	AU 2001-90926	20010912
EP 1317474	A2	20030611	EP 2001-970986	20010912
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				

PRIORITY APPLN. INFO.:
US 2000-660587 A 20000912
US 1998-201458 A2 19981130
US 1999-261358 A2 19990303
WO 2001-US28759 W 20010912

AB The present invention is directed to the cloning, sequencing and expression of homologous immunoreactive **28-kDa** outer membrane protein genes, **p28**-1, -2, -3, -5, -6, -7, -9, from a polymorphic multiple gene family of **Ehrlichia canis**. Further disclosed is a multigene locus encoding all nine homologous **28-kDa** protein genes of **Ehrlichia canis**. Recombinant **Ehrlichia canis 28-kDa** proteins react with convalescent phase antiserum from an **E.canis** -infected dog, and may be useful in the development of vaccines and serodiagnostics that are particularly effective for disease prevention and serodiagnosis. The invention also relates to methods and compns. directed toward the prevention of **E. canis** infection of dogs.

L21 ANSWER 3 OF 17 HCAPLUS COPYRIGHT 2003 ACS DUPLICATE 3
ACCESSION NUMBER: 2002:444530 HCAPLUS
DOCUMENT NUMBER: 137:29031
TITLE: Protein and DNA sequences of **Ehrlichia canis** homologous **28-kilodalton** immunodominant protein gene family and uses thereof
INVENTOR(S): **Walker, David H.; Yu, Xue-Jie ; McBride, Jere W.**
PATENT ASSIGNEE(S): Research Development Foundation, USA
SOURCE: U.S., 42 pp., Cont.-in-part of U.S. Ser. No. 201,458.
CODEN: USXXAM

DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 4
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 6403780	B1	20020611	US 1999-261358	19990303
US 6458942	B1	20021001	US 1998-201458	19981130
WO 2000032745	A2	20000608	WO 1999-US28075	19991124
W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
AU 2000019234	A5	20000619	AU 2000-19234	19991124
BR 9916141	A	20011204	BR 1999-16141	19991124
US 6392023	B1	20020521	US 2000-660587	20000912
US 2002115840	A1	20020822	US 2002-62624	20020131
US 2003073095	A1	20030417	US 2002-62051	20020131
US 2003096250	A1	20030522	US 2002-62920	20020131

PRIORITY APPLN. INFO.:

US 1998-201458	A2	19981130
US 1999-261358	A	19990303
WO 1999-US28075	W	19991124
US 2000-660279	A3	20000912
US 2000-660587	A3	20000912

AB The present invention is directed to the cloning, sequencing and expression of homologous immunoreactive **28-kDa** protein genes, ECa28-1, ECaSA2, and ECa28SA3, from a polymorphic multiple gene family of **Ehrlichia canis**. Further disclosed is a multigene locus encoding all five homologous **28-kDa** protein genes of **Ehrlichia canis**, and the five proteins are predicted to have signal peptides resulting in mature proteins and had amino acid homol. ranging from 51 to 72%. Anal. of intergenic regions revealed hypothetical promoter regions for each gene, suggesting that these genes may be independently and differentially expressed. The invention further provides expression vectors comprising genes encoding the **28-kDa** immunoreactive proteins and capable of expressing the gened when the vectors are introduced into cells. The invention discloses that the recombinant **Ehrlichia canis 28-kDa** proteins react with convalescent phase antiserum from an **E. canis**-infected dog.

REFERENCE COUNT: 1 THERE ARE 1 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L21 ANSWER 4 OF 17 HCAPLUS COPYRIGHT 2003 ACS DUPLICATE 4
ACCESSION NUMBER: 2002:387621 HCAPLUS
DOCUMENT NUMBER: 136:381390
TITLE: Protein and DNA sequences of homologous **28-kilodalton** immunodominant protein genes of **Ehrlichia**

canis and therapeutical uses

INVENTOR(S): **Walker, David H.; Yu, Xue-Jie ; McBride, Jere W.**
PATENT ASSIGNEE(S): Research Development Foundation, USA
SOURCE: U.S., 49 pp., Cont.-in-part of U.S. Ser. No. 261,358.
CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 4
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 6392023	B1	20020521	US 2000-660587	20000912
US 6458942	B1	20021001	US 1998-201458	19981130
US 6403780	B1	20020611	US 1999-261358	19990303
WO 2002022782	A2	20020321	WO 2001-US28759	20010912
WO 2002022782	A3	20020530		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
AU 2001090926	A5	20020326	AU 2001-90926	20010912
EP 1317474	A2	20030611	EP 2001-970986	20010912
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				
US 2003073095	A1	20030417	US 2002-62051	20020131
US 2003096250	A1	20030522	US 2002-62920	20020131

PRIORITY APPLN. INFO.:
US 1998-201458 A2 19981130
US 1999-261358 A2 19990303
US 2000-660587 A 20000912
WO 2001-US28759 W 20010912

AB The present invention is directed to the cloning, sequencing and expression of homologous immunoreactive **28-kDa** protein genes, **p28**-1, -2, -3, -5, -6, -7, -9, from a polymorphic multiple gene family of **Ehrlichia canis**. Further disclosed is a multigene locus encoding all nine homologous **28-kDa** protein genes of **Ehrlichia canis**. The invention also provides expression vectors comprising genes encoding the **28-kDa** proteins which are capable of expressing the recombinant proteins when the vectors are introduced into a cell. The **Ehrlichia canis 28-kDa** proteins react with convalescent phase antiserum from an **E. canis**-infected dog, and may be useful in the development of vaccines and serodiagnostics that are particularly effective for disease prevention and serodiagnosis.

REFERENCE COUNT: 3 THERE ARE 3 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L21 ANSWER 5 OF 17 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2002:609790 BIOSIS
DOCUMENT NUMBER: PREV200200609790
TITLE: **28-kDa** immunoreactive protein gene of **Ehrlichia canis** and uses thereof.
AUTHOR(S): **Walker, David H.; McBride, Jere W. (1); Yu, Xue-Jie**
CORPORATE SOURCE: (1) Galveston, TX USA
ASSIGNEE: Research Development Foundation, Alexandria, VA, USA
PATENT INFORMATION: US 6458942 October 01, 2002
SOURCE: Official Gazette of the United States Patent and Trademark Office Patents, (Oct. 1, 2002) Vol. 1263, No. 1, pp. No Pagination.
http://www.uspto.gov/web/menu/patdata.html. e-file.
ISSN: 0098-1133.
DOCUMENT TYPE: Patent
LANGUAGE: English
AB The present invention is directed to the cloning, sequencing and expression of a conserved immunoreactive **28-kDa** protein gene (**P28**) from a polymorphic multiple gene family of **Ehrlichia canis**. **E. canis** **P28** has an 834-bp open reading frame encoding a protein of 278 amino acids with four variable regions, and shares similar surface-exposed regions, antigenicity and T-cell motifs with E. chaffeensis **P28**. Also disclosed is that recombinant **E. canis P28** protein reacts with convalescent phase antiserum from an **E. canis** -infected dog.

L21 ANSWER 6 OF 17 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2002:278689 BIOSIS
DOCUMENT NUMBER: PREV200200278689
TITLE: P43 antigen for the immunodiagnosis of canine ehrlichiosis and uses thereof.
AUTHOR(S): **Walker, David H. (1); McBride, Jere W.**
CORPORATE SOURCE: (1) Galveston, TX USA
ASSIGNEE: Research Development Foundation
PATENT INFORMATION: US 6355777 March 12, 2002
SOURCE: Official Gazette of the United States Patent and Trademark Office Patents, (Mar. 12, 2002) Vol. 1256, No. 2, pp. No Pagination.
http://www.uspto.gov/web/menu/patdata.html. e-file.
ISSN: 0098-1133.
DOCUMENT TYPE: Patent
LANGUAGE: English
AB Canine monocytic ehrlichiosis, caused by Ehrilichia canis is a potentially fatal disease of dogs that requires rapid and accurate diagnosis in order to initiate appropriate therapy leading to a favorable prognosis. In the invention described herein, a new immunoreactive **E. canis** surface protein gene of 1170-bp was cloned, which encodes a protein with a predicted molecular mass of 42.6 kilodaltons (P43). The P43 gene was not found in E. chaffeensis DNA by Southern blot, and antisera against recombinant P43 (rP43) did not react with E. chaffeensis by IFA. The

P43 was located on the surface of **E. canis** by immunoelectron microscopy. Forty-two dogs exhibiting signs and/or hematologic abnormalities associated with canine ehrlichiosis were tested by IFA and by Western immunoblot. Among the 22 samples that were IFA positive for **E. canis**, 100% reacted with the rP43, 96% with the rP28, and 96% with the rP140. The specificity of the recombinant proteins compared to IFA was 96% for rP28, 88% for P43 and 63% for P140. Results of this study demonstrate that the rP43 and rP28 are sensitive and reliable serodiagnostic antigens for the diagnosis of **Ehrlichia canis** infections.

L21 ANSWER 7 OF 17 HCAPLUS COPYRIGHT 2003 ACS DUPLICATE 5
ACCESSION NUMBER: 2001:816394 HCAPLUS
DOCUMENT NUMBER: 135:356748
TITLE: P43 antigen for the immunodiagnosis of canine ehrlichiosis and uses thereof
INVENTOR(S): **Walker, David H.; McBride, Jere W.**
PATENT ASSIGNEE(S): Research Development Foundation, USA
SOURCE: PCT Int. Appl., 60 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001082862	A2	20011108	WO 2001-US13446	20010427
WO 2001082862	A3	20020404		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
US 6355777	B1	20020312	US 2000-561322	20000428
AU 2001055702	A5	20011112	AU 2001-55702	20010427
EP 1276492	A2	20030122	EP 2001-928896	20010427
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				

PRIORITY APPLN. INFO.: US 2000-561322 A 20000428
WO 2001-US13446 W 20010427

AB Canine monocytic ehrlichiosis, caused by **Ehrlichia canis** is a potentially fatal disease of dogs that requires rapid and accurate diagnosis in order to initiate appropriate therapy leading to a favorable prognosis. In the invention described herein, a new immunoreactive **E. canis** surface protein gene of 1170-bp was cloned, which encodes a protein with a predicted mol. mass of 42.6 kilodaltons (P43). The P43 gene was not found in E. chaffeensis DNA by Southern blot, and antisera against recombinant P43 (rP43) did not react with E. chaffeensis by

indirect fluorescent antibody (IFA). The P43 was located on the surface of **E. canis** by immunoelectron microscopy. Forty-two dogs exhibiting signs and/or hematol. abnormalities assocd. with canine ehrlichiosis were tested by IFA and by Western immunoblot. Among the 22 samples that were IFA pos. for **E. canis**, 100 reacted with the rP43, 96 with the rP28, and 96 with the rP140. The specificity of the recombinant proteins compared to IFA was 96 for rp28, 88 for P43 and 63 for P140. Results of this study demonstrate that the rP43 and rP28 are sensitive and reliable serodiagnostic antigens for the diagnosis of **Ehrlichia canis** infections.

L21 ANSWER 8 OF 17 SCISEARCH COPYRIGHT 2003 THOMSON ISI
ACCESSION NUMBER: 2001:771051 SCISEARCH
THE GENUINE ARTICLE: 474FT
TITLE: Serologic and molecular evidence of coinfection with multiple vector-borne pathogens in dogs from Thailand
AUTHOR: Suksawat J; **Yu X J**; Hancock S I; Hegarty B C; Nilkumhang P; Breitschwerdt E B (Reprint)
CORPORATE SOURCE: N Carolina State Univ, Coll Vet Med, Dept Clin Sci, 4700 Hillsborough St, Raleigh, NC 27606 USA (Reprint); N Carolina State Univ, Coll Vet Med, Dept Clin Sci, Raleigh, NC 27606 USA; Khon Kaen Univ, Fac Vet Med, Dept Vet Med, Khon Kaen, Thailand; Univ Texas, Med Branch, Sch Med, Dept Pathol, Galveston, TX 77550 USA; Kasetsart Univ, Fac Vet Med, Dept Small Anim Med, Bangkok, Thailand
COUNTRY OF AUTHOR: USA; Thailand
SOURCE: JOURNAL OF VETERINARY INTERNAL MEDICINE, (SEP-OCT 2001) Vol. 15, No. 5, pp. 453-462.
Publisher: AMER COLL VETERINARY INTERNAL MEDICINE, 7175 W JEFFERSON AVE, STE 2125, LAKEWOOD, CO 80235 USA.
ISSN: 0891-6640.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 68

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Forty-nine dogs from Thailand were evaluated for serologic evidence of exposure or polymerase chain reaction (PCR) evidence of infection with vectorborne pathogens, including Ehrlichia sp. (**Ehrlichia canis**, Ehrlichia chaffeensis, Ehrlichia equi, and Ehrlichia risticii). Bartonella vinsonii subsp. berkhoffi (Bvb), spotted fever group (SFG) rickettsiae (Rickettsia rickettsii), Typhus group (TG) rickettsiae (Rickettsia canada, Rickettsia prowazekii. and Rickettsia typhi). and Babesia sp. (Bahesia canis and Babesia gibsonii). All study dogs had at least I of 3 entry criteria: fever, anemia, or thrombocytopenia, By immunofluorescence antibody (IFA) testing, seroreactivity was most prevalent to E chaffeensis (74%) and E cams (71%) antigens, followed by E equi (.58%), Bvb (38%), E risticii (38%). R prowazekii (24%), B canis (20%). R rickettsii (12%), R canada (4%), and B gibsonii (4%) antigens. There was 100% concordance between **E canis** IFA and western blot immunoassay (WI) for 35 of 35 samples 2 samples were IFA and WI reactive only to E equi antigens. By PCR amplification, 10 dogs were found to be infected with **E canis**, 5 with Ehrlichia platys, and 3 with B

canis. Sequencing of PCR products was undertaken to compare Ehrlichia strains from Thailand to strains originating from the United States. Partial DNA sequence analysis confirmed infection with **E canis** and E platys, with identical 16S rRNA sequence alignment to **E canis** (U26740) and to E platys (M83801), as reported in GenBank. Partial **E canis P28**.1 and **P28**.2 amino acid sequences from Thai dogs were divergent from analogous sequences derived from North American **E canis** (AF082744) strains, suggesting that the Thai dogs were infected with a geographically distinct strain of **E canis** compared to North American strains. The results of this study indicate that dogs in Thailand have substantial exposure to vectorborne diseases and that coinfection with these pathogens may be common.

L21 ANSWER 9 OF 17 HCAPLUS COPYRIGHT 2003 ACS DUPLICATE 6
ACCESSION NUMBER: 2001:83487 HCAPLUS
DOCUMENT NUMBER: 134:350187
TITLE: Immunodiagnosis of **Ehrlichia canis** infection with recombinant proteins
AUTHOR(S): **McBride, Jere W.**; Corstvet, Richard E.; Breitschwerdt, Edward B.; **Walker, David H.**
CORPORATE SOURCE: Department of Pathology and WHO Collaborating Center for Tropical Diseases, University of Texas Medical Branch, Galveston, TX, 77555, USA
SOURCE: Journal of Clinical Microbiology (2001), 39(1), 315-322
CODEN: JCMIDW; ISSN: 0095-1137
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB **Ehrlichia canis** causes a potentially fatal rickettsial disease of dogs that requires rapid and accurate diagnosis in order to initiate appropriate therapy leading to a favorable prognosis. We recently reported the cloning of two immunoreactive **E. canis** proteins, **P28** and P140, that were applicable for serodiagnosis of the disease. In the present study we cloned a new immunoreactive **E. canis** surface protein gene of 1,170 bp, which encodes a protein with a predicted mol. mass of 42.6 kDa (P43). The P43 gene was not detected in E. chaffeensis DNA by Southern blot, and antisera against recombinant P43 (rP43) did not react with E. chaffeensis as detected by indirect fluorescent antibody (IFA) assay. Forty-two dogs exhibiting signs and/or hematol. abnormalities assocd. with canine ehrlichiosis were tested by IFA assay and by recombinant Western immunoblot. Among the 22 samples that were IFA pos. for **E. canis**, 100% reacted with rP43, 96% reacted with rP28, and 96% reacted with rP140. The specificity of the recombinant proteins compared to the IFAs was 96% for rP28, 88% for P43 and 63% for P140. The results of this study demonstrate that the rP43 and rP28 are sensitive and reliable serodiagnostic antigens for **E. canis** infections.

REFERENCE COUNT: 30 THERE ARE 30 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L21 ANSWER 10 OF 17 HCAPLUS COPYRIGHT 2003 ACS DUPLICATE 7
ACCESSION NUMBER: 2000:384370 HCAPLUS
DOCUMENT NUMBER: 133:27381
TITLE: Sequences of two novel homologous **28-kilodalton** immunodominant protein genes (ECa28-1 and ECa28SA3) of **Ehrlichia canis** and uses thereof
INVENTOR(S): **Walker, David H.; Yu, Xue-jie ; McBride, Jere W.**
PATENT ASSIGNEE(S): Research Development Foundation, USA
SOURCE: PCT Int. Appl., 86 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 4
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000032745	A2	20000608	WO 1999-US28075	19991124
W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
US 6458942	B1	20021001	US 1998-201458	19981130
US 6403780	B1	20020611	US 1999-261358	19990303
AU 2000019234	A5	20000619	AU 2000-19234	19991124
BR 9916141	A	20011204	BR 1999-16141	19991124

PRIORITY APPLN. INFO.:
US 1998-201458 A 19981130
US 1999-261358 A 19990303
WO 1999-US28075 W 19991124

AB The invention provides sequences of two novel homologous immunoreactive **28-kDa** protein genes, ECa28-1 and ECa28SA3, from a polymorphic multiple gene family of **Ehrlichia canis**. A complete sequence of another **28-kDa** protein gene, ECa28SA2, which was previously only partially sequenced, is also provided. Further disclosed is a multigene locus (5.592-kb) encoding all five homologous **28-kDa** outer membrane protein genes (ECa28SA1, ECa28SA2, ECa28SA3, ECa28-1, and ECa28-2). Recombinant **Ehrlichia canis 28-kDa** proteins react with convalescent phase antiserum from an **E. canis**-infected dog. The invention also relates to methods and compns. directed toward the prevention of **E. canis** infection of dogs.

L21 ANSWER 11 OF 17 HCAPLUS COPYRIGHT 2003 ACS DUPLICATE 8
ACCESSION NUMBER: 2000:615928 HCAPLUS
DOCUMENT NUMBER: 134:81644
TITLE: A conserved, transcriptionally active **p28** multigene locus of **Ehrlichia canis**

AUTHOR(S): **McBride, J. W.; Yu, X.-j.; Walker, D. H.**
CORPORATE SOURCE: Department of Pathology and WHO Collaborating Center for Tropical Diseases, University of Texas Medical Branch, Galveston, TX, 77555-0609, USA
SOURCE: Gene (2000), 254(1,2), 245-252
CODEN: GENED6; ISSN: 0378-1119
PUBLISHER: Elsevier Science B.V.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Antigenic diversity of Ehrlichia chaffeensis and **Ehrlichia canis** may involve independent or differential expression of the **P28** outer membrane proteins genes, enabling persistent infections of the natural hosts. In this study, we analyzed the transcriptional activity of a five gene locus in **E. canis** encoding homologous, but non-identical, **p28** genes. The **p28** multigene locus contained three previously identified complete gene sequences and one partial gene sequence. A new **p28** gene was identified and sequenced, and the complete sequence of a second partial **p28** gene was detd. The new **p28** gene joined two previously sep. loci, forming the single **p28** multigene locus. The amino acid homol. of the **E. canis P28** proteins ranged from 51 to 74%. The nucleic acid sequence of regions compared within the locus spanning four **p28** genes from two geog. distinct **E. canis** isolates was completely conserved. Anal. of the five **p28** genes demonstrated that all were transcriptionally active in in-vitro cultures of **E. canis** incubated at the vertebrate host (37.degree.C) and ambient tick temps. (27.degree.C). Polycistronic copies of multiple **p28** genes were not detected by RT-PCR, but monocistronic **p28** mRNA transcripts were detected by Northern blotting from **E. canis** infected DH82 cells, indicating that the genes are transcribed as monocistronic messages.
REFERENCE COUNT: 22 THERE ARE 22 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L21 ANSWER 12 OF 17 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2001:3781 BIOSIS
DOCUMENT NUMBER: PREV200100003781
TITLE: Multiple **Ehrlichia canis** **p28** genes are transcriptionally active as monocistronic messages.
AUTHOR(S): **McBride, J. W. (1); Yu, X.-J. (1) ; Walker, D. H. (1)**
CORPORATE SOURCE: (1) Department of Pathology, University of Texas Medical Branch, Galveston, TX USA
SOURCE: American Journal of Tropical Medicine and Hygiene, (March, 2000) Vol. 62, No. 3 Supplement, pp. 188. print.
Meeting Info.: 49th Annual Meeting of the American Society of Tropical Medicine and Hygiene Houston, Texas, USA October 29-November 02, 2000 American Society of Tropical Medicine and Hygiene
. ISSN: 0002-9637.
DOCUMENT TYPE: Conference

LANGUAGE: English
SUMMARY LANGUAGE: English

L21 ANSWER 13 OF 17 CABA COPYRIGHT 2003 CABI
ACCESSION NUMBER: 1999:143644 CABA
DOCUMENT NUMBER: 990506067
TITLE: Comparison of Ehrlichia chaffeensis recombinant proteins for serologic diagnosis of human monocytotropic ehrlichiosis
AUTHOR: **Yu XueJie**; Crocquet-Valdes, P. A.; Cullman, L. C.; Popov, V. L.; **Walker, D. H.**; **Yu, X. J.**
CORPORATE SOURCE: Department of Pathology, 301 University Blvd., University of Texas Medical Branch, Galveston, TX 77555-0609, USA.
SOURCE: Journal of Clinical Microbiology, (1999) Vol. 37, No. 8, pp. 2568-2575. 38 ref.
ISSN: 0095-1137
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Protein immunoblotting was used to evaluate the reaction of the antibodies in patients' sera with the recombinant E. chaffeensis 120- and **28-kDa** proteins as well as the 106- and the 37-kDa proteins. The cloning of the genes encoding the latter 2 proteins is described. Immunoelectron microscopy demonstrated that the 106-kDa protein is located at the surfaces of ehrlichiae and on the intramorular fibrillar structures associated with E. chaffeensis. The 37-kDa protein is homologous to the iron-binding protein of Gram-negative bacteria. 42 serum samples from patients who were suspected to have human monocytotropic ehrlichiosis were tested by immunofluorescence (IFA) using E. chaffeensis antigen and by protein immunoblotting using recombinant E. chaffeensis proteins expressed in Escherichia coli. 32 serum samples contained IFA antibodies at a titre of 1:64 or greater. The correlation of IFA and recombinant protein immunoblotting was 100% for the 120-kDa protein, 41% for the **28-kDa** protein, 9.4% for the 106-kDa protein, and 0% for the 37-kDa protein. None of the recombinant antigens yielded false-positive results. All the sera reactive with the recombinant 28- or the 106-kDa proteins also reacted with the recombinant 120-kDa protein. The EMBL\GenBank\DDBJ accession number for the nucleotide sequences of the 106- and 37-kDa protein genes is AF117273.

L21 ANSWER 14 OF 17 HCAPLUS COPYRIGHT 2003 ACS DUPLICATE 9
ACCESSION NUMBER: 1999:336049 HCAPLUS
DOCUMENT NUMBER: 131:165989
TITLE: Molecular cloning of the gene for a conserved major immunoreactive **28-kilodalton** protein of **Ehrlichia canis**: a potential serodiagnostic antigen
AUTHOR(S): **McBride, Jere W.**; **Yu, Xue-Jie**; **Walker, David H.**
CORPORATE SOURCE: Department of Pathology and WHO Collaborating Center for Tropical Diseases, University of Texas Medical Branch, Galveston, TX, 77555-0609, USA
SOURCE: Clinical and Diagnostic Laboratory Immunology

(1999), 6(3), 392-399
CODEN: CDIMEN; ISSN: 1071-412X
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A gene encoding a **28-kDa** protein of **Ehrlichia canis** was cloned, sequenced, and expressed, and a comparative mol. anal. with homologous genes of **E. canis**, Cowdria ruminantium, and Ehrlichia chaffeensis was performed. The complete gene has an 834-bp open reading frame encoding a protein of 278 amino acids with a predicted mol. mass of 30.5 kDa. An N-terminal signal sequence was identified, suggesting that the protein undergoes posttranslational modification to a mature 27.7-kDa protein (**P28**). The **E. canis p28** gene has significant nucleic acid and amino acid sequence homologies with the E. chaffeensis outer membrane protein-1 (omp-1) gene family, with the Cowdria ruminantium map-1 gene, and with other **E. canis 28-kDa**-protein genes. Southern blotting revealed the presence of at least two addnl. homologous **p28** gene copies in the **E. canis** genome, confirming that **p28** is a member of a polymorphic multiple-gene family. Amino acid sequence anal. revealed that **E. canis P28** has four variable regions, and it shares similar surface-exposed regions, antigenicity, and T-cell motifs with E. chaffeensis **P28**. The **p28** genes from seven different **E. canis** isolates were identical, indicating that the gene for this major immunoreactive protein is highly conserved. In addn., reactivity of sera from clin. cases of canine ehrlichiosis with the recombinant **P28** demonstrated that the recombinant protein may be a reliable serodiagnostic antigen.

REFERENCE COUNT: 33 THERE ARE 33 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L21 ANSWER 15 OF 17 HCAPLUS COPYRIGHT 2003 ACS DUPLICATE 10
ACCESSION NUMBER: 1997:767892 HCAPLUS
DOCUMENT NUMBER: 128:33472
TITLE: Western immunoblotting analysis of the antibody responses of patients with human monocytotropic ehrlichiosis to different strains of Ehrlichia chaffeensis and **Ehrlichia canis**
AUTHOR(S): Chen, Sheng-Min; Cullman, Louis C.; **Walker, David H.**
CORPORATE SOURCE: Department of Pathology, University of Texas Medical Branch, Galveston, TX, 77555-0609, USA
SOURCE: Clinical and Diagnostic Laboratory Immunology (1997), 4(6), 731-735
CODEN: CDIMEN; ISSN: 1071-412X
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB To evaluate the relative sensitivity of the detection of antibodies against various antigenic proteins of E. chaffeensis for the diagnosis of the emerging infectious disease human monocytotropic ehrlichiosis, Western immunoblotting was performed with 27 serum

samples from convalescent patients with antibodies, as demonstrated by indirect immunofluorescence assay. Among 22 patients with antibodies reactive with the 120-kDa protein, 15 showed reactivity with the 29/**28-kDa** protein(s) and the proteins in the 44-88-kDa range. Two of the serum samples with this pattern reacted with the 29/**28-kDa** protein(s) of only the 91HE17 strain, and one sample reacted with only that of the Arkansas strain, indicating that the antibodies were stimulated by strain-specific epitopes. Overall, antibodies to the 29/**28 -kDa** protein(s) were detected in only 16 patients' sera, suggesting that this protein is less sensitive than the 120-kDa protein. Two of 12 serum samples from healthy blood donors had antibodies reactive with the 120-kDa protein; one of these samples reacted also with the 29/**28-kDa** protein(s) of **E. canis**, suggesting that unrecognized ehrlichial infection might have occurred, including human infection with **E. canis**. A high correlation between reactivity with the 120-kDa protein by Western immunoblotting and the recombinant 120-kDa protein by dot blot supports the potential usefulness of this recombinant antigen in diagnostic serol.

L21 ANSWER 16 OF 17 HCAPLUS COPYRIGHT 2003 ACS DUPLICATE 11
ACCESSION NUMBER: 1996:305975 HCAPLUS
DOCUMENT NUMBER: 125:29500
TITLE: Analysis and ultrastructural localization of Ehrlichia chaffeensis proteins with monoclonal antibodies
AUTHOR(S): Chen, Sheng-Min; Popov, Vsevolod L.; Feng, Hui-Min; **Walker, David H.**
CORPORATE SOURCE: Department Pathology, University Texas Medical Branch, Galveston, TX, USA
SOURCE: American Journal of Tropical Medicine and Hygiene (1996), 54(4), 405-412
CODEN: AJTHAB; ISSN: 0002-9637
PUBLISHER: American Society of Tropical Medicine and Hygiene
DOCUMENT TYPE: Journal
LANGUAGE: English

AB To det. the nature and ultrastructural location of E. chaffeensis antigens, monoclonal antibodies (MAbs) to E. chaffeensis were developed. The MAbs were used for immunofluorescence and Western immunoblotting anal. of the antigens of d. gradient-purified ehrlichiae. Monoclonal antibody 6A1 recognized an epitope of a 30-kD protein. This antibody reacted with a strain-specific epitope of E. chaffeensis, Arkansas strain, and did not cross-react with any other ehrlichia tested. Monoclonal antibodies 3C7 and 7C1-B recognized Arkansas strain proteins of 30 and 29 kD and reacted with E. chaffeensis (strain 91HE17) proteins of 31 and 29 kD and an **E. canis** protein of 30 kD. Lack of reactivity of these 2 MAbs with E. sennetsu and E. risticii suggests that the epitope is group specific. Monoclonal antibody 5D11 recognized a 58-kD protein of both strains of E. chaffeensis as well as **E . canis**, apparently a group-specific, conformation-independent epitope. Monoclonal antibody 7C1-C reacted with 58- and 88-kD proteins of both the Arkansas and 91HE17 strains. Trypsin treatment destroyed the reactivity of E. chaffeensis antigens with all the MAbs when tested by Western immunoblotting, indicating that these antigens are proteins with trypsin-sensitive epitopes.

Immunoelectron microscopy of neg. stained intact E. chaffeensis organisms showed that the 30- and 29-kD proteins are present on the surface of the ehrlichial cell wall along with the previously localized **28-kD** protein.

L21 ANSWER 17 OF 17 CONFSCI COPYRIGHT 2003 CSA
ACCESSION NUMBER: 2000:70499 CONFSCI
DOCUMENT NUMBER: 00-067370
TITLE: Multiple **Ehrlichia canis** **P28** genes are transcriptionally active as monocistronic messages
AUTHOR: **McBride, J.W.; Yu, X.-J.; Walker, D.H.**
CORPORATE SOURCE: Dep. Pathol., Univ. Texas Med. Branch, Galveston, TX, USA
SOURCE: American Society of Tropical Medicine and Hygiene, 3088 Briarcliff Rd., Ste. 11A, Atlanta, GA 30329, USA

Meeting Info.: 000 5172: ASTMH 49th Annual Meeting (0005172). Houston, Texas (USA). 29 Oct-2 Nov 2000. American Society of Tropical Medicine and Hygiene.
DOCUMENT TYPE: Conference
FILE SEGMENT: DCCP
LANGUAGE: English

FILE 'HOME' ENTERED AT 10:56:43 ON 09 JUL 2003

-key terms

FILE 'USPATFULL' ENTERED AT 12:12:46 ON 09 JUL 2003
L1 116 SEA FILE=HCAPLUS ABB=ON PLU=ON (EHRLICH? OR E)(W)CANIS

L22 15 SEA FILE=USPATFULL ABB=ON PLU=ON L1(S)(28KD? OR 28KILOD? OR 28(W)(KD? OR KILOD? OR KILO(W)(D OR DA OR DALTON)) OR P28)

L22 ANSWER 1 OF 15 USPATFULL
ACCESSION NUMBER: 2003:173238 USPATFULL
TITLE: Compositions and methods for detection of Ehrlichia canis and Ehrlichia chaffeensis antibodies
INVENTOR(S): Lawton, Robert, Gorham, ME, UNITED STATES
O'Connor, Thomas Patrick, JR., Westbrook, ME, UNITED STATES
Bartol, Barbara Ann, Gorham, ME, UNITED STATES
MacHenry, Paul Scott, Portland, ME, UNITED STATES
PATENT ASSIGNEE(S): IDEXX Laboratories (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2003119082	A1	20030626
APPLICATION INFO.:	US 2002-54354	A1	20020122 (10)

RELATED APPLN. INFO.: Division of Ser. No. US 2001-765739, filed on 18 Jan 2001, PENDING
DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: MCDONNELL BOEHNEN HULBERT & BERGHOFF, 300 SOUTH WACKER DRIVE, SUITE 3200, CHICAGO, IL, 60606
NUMBER OF CLAIMS: 6
EXEMPLARY CLAIM: 1
LINE COUNT: 731
AB The invention provides methods and compositions for the detection of Ehrlichia canis and Ehrlichia chaffeensis antibodies and antibody fragments.

INCL INCLM: 435/007.320
NCL NCLM: 435/007.320

L22 ANSWER 2 OF 15 USPATFULL
ACCESSION NUMBER: 2003:140409 USPATFULL
TITLE: Homologous **28-kilodalton** immunodominant protein genes of **Ehrlichia canis** and uses thereof
INVENTOR(S): Walker, David H., Galveston, TX, UNITED STATES
Yu, Xue-Jie, Houston, TX, UNITED STATES
McBride, Jere W., Galveston, TX, UNITED STATES

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2003096250	A1	20030522
APPLICATION INFO.:	US 2002-62920	A1	20020131 (10)

RELATED APPLN. INFO.: Division of Ser. No. US 2000-660587, filed on 12 Sep 2000, GRANTED, Pat. No. US 6392023
Continuation-in-part of Ser. No. US 1999-261358, filed on 3 Mar 1999, GRANTED, Pat. No. US 6403780
Continuation-in-part of Ser. No. US 1998-201458, filed on 30 Nov 1998, GRANTED, Pat. No. US

6458942
DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: Benjamin Aaron Adler, Ph.D., J.D., Adler & Associates, 8011 Candle Lane, Houston, TX, 77071
NUMBER OF CLAIMS: 20
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 20 Drawing Page(s)
LINE COUNT: 2208
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention is directed to the cloning, sequencing and expression of homologous immunoreactive **28-kDa** protein genes, **p28**-1, -2, -3, -5, -6, -7, -9, from a polymorphic multiple gene family of **Ehrlichia canis**. Further disclosed is a multigene locus encoding all nine homologous **28-kDa** protein genes of **Ehrlichia canis**. Recombinant **Ehrlichia canis 28-kDa** proteins react with convalescent phase antiserum from an **E. canis** -infected dog, and may be useful in the development of vaccines and serodiagnostics that are particularly effective for disease prevention and serodiagnosis.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
INCL INCLM: 435/006.000
INCLS: 435/069.300; 435/252.300; 435/320.100; 530/350.000; 536/023.700
NCL NCLM: 435/006.000
NCLS: 435/069.300; 435/252.300; 435/320.100; 530/350.000; 536/023.700

L22 ANSWER 3 OF 15 USPATFULL
ACCESSION NUMBER: 2003:133503 USPATFULL
TITLE: Ehrlichia chaffeensis 28 kDa outer membrane protein multigene family
INVENTOR(S): Walker, David H., Galveston, TX, UNITED STATES
Yu, Xue-Jie, Galveston, TX, UNITED STATES
PATENT ASSIGNEE(S): Research Development Foundation (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2003091588	A1	20030515
APPLICATION INFO.:	US 2002-284986	A1	20021031 (10)

RELATED APPLN. INFO.: Division of Ser. No. US 2001-846808, filed on 1 May 2001, PENDING

	NUMBER	DATE
PRIORITY INFORMATION:	US 2000-201035P	20000501 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: Benjamin Aaron Adler, Adler & Associates, 8011 Candle Lane, Houston, TX, 77071
NUMBER OF CLAIMS: 6
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 8 Drawing Page(s)
LINE COUNT: 2073

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The 28-kDa outer membrane proteins (P28) of Ehrlichia chaffeensis are encoded by a multigene family consisting of 21 members located in a 23-kb DNA fragment in the genome of E. chaffeensis. Fifteen of these proteins are claimed herein as novel sequences. The amino acid sequence identity of the various P28 proteins was 20-83%. Six of 10 tested p28 genes were actively transcribed in cell culture grown E. chaffeensis. RT-PCR also indicated that each of the p28 genes was monocistronic. These results suggest that the p28 genes are active genes and encode polymorphic forms of the P28 proteins. The P28s were also divergent among different isolates of E. chaffeensis. The large repertoire of the p28 genes in a single ehrlichial organism and antigenic diversity of the P28 among the isolates of E. chaffeensis suggest that the P28s may be involved in immune avoidance.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
INCL INCLM: 424/190.100
INCLS: 530/350.000
NCL NCLM: 424/190.100
NCLS: 530/350.000

L22 ANSWER 4 OF 15 USPATFULL
ACCESSION NUMBER: 2003:106184 USPATFULL
TITLE: Homologous **28-kilodalton** immunodominant protein genes of **Ehrlichia canis** and uses thereof
INVENTOR(S): Walker, David H., Galveston, TX, UNITED STATES
Yu, Xue-Jie, Houston, TX, UNITED STATES
McBride, Jere W., Galveston, TX, UNITED STATES

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2003073095	A1	20030417
APPLICATION INFO.:	US 2002-62051	A1	20020131 (10)

RELATED APPLN. INFO.: Division of Ser. No. US 2000-660587, filed on 12 Sep 2000, GRANTED, Pat. No. US 6392023
Continuation-in-part of Ser. No. US 1999-261358, filed on 3 Mar 1999, GRANTED, Pat. No. US 6403780
Continuation-in-part of Ser. No. US 1998-201458, filed on 30 Nov 1998, GRANTED, Pat. No. US 6458942
DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: Benjamin Aaron Adler, Ph.D., J.D., Adler & Associates, 8011 Candle Lane, Houston, TX, 77071
NUMBER OF CLAIMS: 20
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 20 Drawing Page(s)
LINE COUNT: 2207
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention is directed to the cloning, sequencing and expression of homologous immunoreactive **28-kDa** protein genes, **p28**-1, -2, -3, -5; -6, -7, -9, from a polymorphic multiple gene family of **Ehrlichia canis**. Further disclosed is a multigene locus encoding all nine homologous **28-kDa** protein genes of **Ehrlichia canis**. Recombinant **Ehrlichia**

canis 28-kDa proteins react with convalescent phase antiserum from an **E. canis** -infected dog, and may be useful in the development of vaccines and serodiagnostics that are particularly effective for disease prevention and serodiagnosis.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
INCL INCLM: 435/006.000
INCLS: 435/069.300; 435/252.300; 435/320.100; 530/350.000; 536/023.700
NCL NCLM: 435/006.000
NCLS: 435/069.300; 435/252.300; 435/320.100; 530/350.000; 536/023.700

L22 ANSWER 5 OF 15 USPATFULL
ACCESSION NUMBER: 2002:314701 USPATFULL
TITLE: Compositions and methods for detection of ehrlichia canis and ehrlichia chaffeensis antibodies
INVENTOR(S): Lawton, Robert, Gorham, ME, UNITED STATES
O'Connor, Thomas Patrick, JR., Westbrook, ME, UNITED STATES
Bartol, Barbara Ann, Gorham, ME, UNITED STATES
MacHenry, Paul Scott, Portland, ME, UNITED STATES

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2002177178	A1	20021128	
APPLICATION INFO.:	US 2001-765739	A1	20010118	(9)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: MCDONNELL BOEHNEN HULBERT & BERGHOFF, 300 SOUTH WACKER DRIVE, SUITE 3200, CHICAGO, IL, 60606
NUMBER OF CLAIMS: 34
EXEMPLARY CLAIM: 1
LINE COUNT: 802
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention provides methods and compositions for the detection of Ehrlichia canis and Ehrlichia chaffeensis antibodies and antibody fragments.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
INCL INCLM: 435/007.320
INCLS: 530/388.400
NCL NCLM: 435/007.320
NCLS: 530/388.400

L22 ANSWER 6 OF 15 USPATFULL
ACCESSION NUMBER: 2002:287569 USPATFULL
TITLE: Compositions and methods for detection of Ehrlichia canis and Ehrlichia chaffeensis antibodies
INVENTOR(S): Lawton, Robert, Gorham, ME, UNITED STATES
O'Connor, Thomas Patrick, JR., Westbrook, ME, UNITED STATES
Bartol, Barbara Ann, Gorham, ME, UNITED STATES
MacHenry, Paul Scott, Portland, ME, UNITED STATES
PATENT ASSIGNEE(S): IDEXX Laboratories. (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2002160432	A1	20021031
APPLICATION INFO.:	US 2002-54647	A1	20020122 (10)

RELATED APPLN. INFO.: Division of Ser. No. US 2001-765739, filed on 18 Jan 2001, PENDING
DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: MCDONNELL BOEHNEN HULBERT & BERGHOFF, 300 SOUTH WACKER DRIVE, SUITE 3200, CHICAGO, IL, 60606
NUMBER OF CLAIMS: 6
EXEMPLARY CLAIM: 1
LINE COUNT: 732
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention provides methods and compositions for the detection of Ehrlichia canis and Ehrlichia chaffeensis antibodies and antibody fragments.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
INCL INCLM: 435/007.320
INCLS: 530/388.400
NCL NCLM: 435/007.320
NCLS: 530/388.400

L22 ANSWER 7 OF 15 USPATFULL
ACCESSION NUMBER: 2002:254464 USPATFULL
TITLE: **28-kDa** immunoreactive protein gene of **Ehrlichia canis** and uses thereof
INVENTOR(S): Walker, David H., Galveston, TX, United States
McBride, Jere W., Galveston, TX, United States
Yu, Xue-Jie, Galveston, TX, United States
PATENT ASSIGNEE(S): Research Development Foundation, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6458942	B1	20021001
APPLICATION INFO.:	US 1998-201458		19981130 (9)

DOCUMENT TYPE: Utility
FILE SEGMENT: GRANTED
PRIMARY EXAMINER: Carlson, Karen Cochrane
ASSISTANT EXAMINER: Robinson, Patricia
LEGAL REPRESENTATIVE: Adler, Benjamin Aaron
NUMBER OF CLAIMS: 7
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 6 Drawing Figure(s); 6 Drawing Page(s)
LINE COUNT: 1723
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention is directed to the cloning, sequencing and expression of a conserved immunoreactive **28-kDa** protein gene (**P28**) from a polymorphic multiple gene family of **Ehrlichia canis**. **E. canis P28** has an 834-bp open reading frame encoding a protein of 278 amino acids with four variable regions, and shares similar surface-exposed regions, antigenicity and T-cell motifs with E. chaffeensis **P28**. Also disclosed is

that recombinant **E. canis P28**
protein reacts with convalescent phase antiserum from an **E**
. canis-infected dog.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
INCL INCLM: 536/023.500
INCLS: 536/023.100; 536/023.500; 435/069.100; 435/252.300; 435/069.800; 435/300.100
NCL NCLM: 536/023.500
NCLS: 435/069.100; 435/069.800; 435/252.300; 435/300.100; 536/023.100

L22 ANSWER 8 OF 15 USPATFULL
ACCESSION NUMBER: 2002:243598 USPATFULL
TITLE: Nucleic acid vaccines against rickettsial diseases and methods of use
INVENTOR(S): Barbet, Anthony F., Archer, FL, UNITED STATES
Bowie, Michael V., Gainesville, FL, UNITED STATES
Ganta, Roman Reddy, Manhattan, KS, UNITED STATES
Burridge, Michael J., Gainesville, FL, UNITED STATES
Mahan, Suman M., Harare, ZIMBABWE
McGuire, Travis C., Pullman, WA, UNITED STATES
Rurangirwa, Fred R., Pullman, WA, UNITED STATES
Moreland, Annie L., Trenton, FL, UNITED STATES
Simbi, Bigboy H., Harare, ZIMBABWE
Whitmire, William M., Hamilton, MT, UNITED STATES
Alleman, Arthur R., Alachua, FL, UNITED STATES

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2002132789	A1	20020919
APPLICATION INFO.:	US 2002-62994	A1	20020131 (10)

RELATED APPLN. INFO.: Division of Ser. No. US 2000-553662, filed on 21 Apr 2000, PENDING Continuation-in-part of Ser. No. US 1999-337827, filed on 22 Jun 1999, PENDING Division of Ser. No. US 1997-953326, filed on 17 Oct 1997, GRANTED, Pat. No. US 6251872 Continuation-in-part of Ser. No. US 1996-733230, filed on 17 Oct 1996, GRANTED, Pat. No. US 6025338

	NUMBER	DATE
PRIORITY INFORMATION:	US 1999-130725P	19990422 (60)
	US 2001-269944P	20010220 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: SALIWANCHIK LLOYD & SALIWANCHIK, A PROFESSIONAL ASSOCIATION, 2421 N.W. 41ST STREET, SUITE A-1, GAINESVILLE, FL, 326066669
NUMBER OF CLAIMS: 20
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 8 Drawing Page(s)
LINE COUNT: 1806
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Described are nucleic acid vaccines containing genes to protect animals or humans against rickettsial diseases. Also described are

polypeptides and methods of using these polypeptides to detect antibodies to pathogens.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
INCL INCLM: 514/044.000
INCLS: 536/023.700
NCL NCLM: 514/044.000
NCLS: 536/023.700

L22 ANSWER 9 OF 15 USPATFULL
ACCESSION NUMBER: 2002:214448 USPATFULL
TITLE: Homologous **28-kilodaltion** immunodominant protein genes of **Ehrlichia canis** and uses thereof
INVENTOR(S): Walker, David H., Galveston, TX, UNITED STATES
Yu, Xue-Jie, Houston, TX, UNITED STATES
McBride, Jere W., Galveston, TX, UNITED STATES

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2002115840	A1	20020822
APPLICATION INFO.:	US 2002-62624	A1	20020131 (10)

RELATED APPLN. INFO.: Division of Ser. No. US 2000-660279, filed on 12 Sep 2000, PENDING Continuation-in-part of Ser. No. US 1999-261358, filed on 3 Mar 1999, GRANTED, Pat. No. US 6403780 Continuation-in-part of Ser. No. US 1998-201458, filed on 30 Nov 1998, ABANDONED
DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: Benjamin Aaron Adler, Ph.D., J.D., Adler & Associates, 8011 Candle Lane, Houston, TX, 77071
NUMBER OF CLAIMS: 20
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 20 Drawing Page(s)
LINE COUNT: 2267
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention is directed to the cloning, sequencing and expression of homologous immunoreactive **28-kDa** protein genes, **p28**-1, -2, -3, -5, -6, -7, -9, from a polymorphic multiple gene family of **Ehrlichia canis**. Further disclosed is a multigene locus encoding all nine homologous **28-kDa** protein genes of **Ehrlichia canis**. Recombinant **Ehrlichia canis 28-kDa** proteins react with convalescent phase antiserum from an **E. canis** -infected dog, and may be useful in the development of vaccines and serodiagnostics that are particularly effective for disease prevention and serodiagnosis.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
INCL INCLM: 536/023.100
INCLS: 435/320.100
NCL NCLM: 536/023.100
NCLS: 435/320.100

L22 ANSWER 10 OF 15 USPATFULL
ACCESSION NUMBER: 2002:201846 USPATFULL

TITLE: Methods for detecting Ehrlichia canis and Ehrlichia chaffeensis in vertebrate and invertebrate hosts

INVENTOR(S): Stich, Roger William, Columbus, OH, United States
Rikihisa, Yasuko, Worthington, OH, United States
PATENT ASSIGNEE(S): The Ohio State University Research Foundation, Columbus, OH, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6432649	B1	20020813
APPLICATION INFO.:	US 2000-648520		20000825 (9)

DOCUMENT TYPE: Utility
FILE SEGMENT: GRANTED
PRIMARY EXAMINER: Yucel, Remy
ASSISTANT EXAMINER: Katcheves, Konstantina
LEGAL REPRESENTATIVE: Calfee, Halter & Griswold LLP
NUMBER OF CLAIMS: 10
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 11 Drawing Figure(s); 11 Drawing Page(s)
LINE COUNT: 1182
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Tools and methods for detecting the presence of **E. canis** and E. chaffeensis in a sample obtained from an animal are provided. The methods employ a polymerase chain reaction and primer sets that are based on the p30 gene of **E. canis** and the **p28** gene of E. chaffeensis. The present invention also relates to the p30 and the **p28** primer sets. Each p30 primer set comprises a first primer and the second primer, both of which are from 15 to 35 nucleotides in length. The first p30 primer comprises a sequence which is complementary to a consecutive sequence, within the following sequence: CCA AGTGTCTCAC ATTTTGGTAG CTTCTCAGCT AAAGAAGAAA GCAAATCAAC TGTTGGAGTTTTTGGATTAA AACATGATTG GGATGGAAGT CCAATACTTA AGAATAAACA CGCTGACTTTACTGTTCCAA AC. SEQ ID NO.1. The second p30 primer comprises a sequence which is complementary to the inverse complement of a consecutive sequence contained within the following sequence: GTTACT CAATGGGTGG CCCAAGAATA GAATTCGAAA TATCTTATGA AGCATTCGAC GTAAAAAGTC CTAATATCAA TTATCAAAAT GACGCGCACA GGTACTGCGC TCTATCTCAT CACACATCGG CAGCCAT, SEQ ID NO.2. The first **p28** comprises a sequence which is complementary to a consecutive sequenc, within the following sequence : A GTTTTCATAA CAAGTGCATT GATATCACTA ATATCTTCTC TACCTGGAGT ATCATTTTCC GACCCAACAG GTAGTGGTAT TAACGG, SEQ ID NO. 3. The second **p28** primer comprises a sequence which is complementary to the inverse complement of a consecutive sequence within one of the following two sequences: CAT TTCTAGGTTT TGCAGGAGCT ATTGGCTACT CAATGGATGG TCCAAGAATA GAGCTTGAAG TATCTTATGA, SEQ ID NO. 4, or C AAGGAAAGTT AGGTTTAAGC TACTCTATAA GCCCAGA, SEQ ID NO. 5.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
INCL INCLM: 435/006.000
INCLS: 536/024.320; 536/024.330
NCL NCLM: 435/006.000
NCLS: 536/024.320; 536/024.330

L22 ANSWER 11 OF 15 USPATFULL
ACCESSION NUMBER: 2002:137155 USPATFULL

TITLE: Homologous **28-kilodalton** immunodominant protein genes of **ehrlichia canis** and uses thereof

INVENTOR(S): Walker, David H., Galveston, TX, United States
Yu, Xue-Jie, Galveston, TX, United States
McBride, Jere W., Galveston, TX, United States
PATENT ASSIGNEE(S): Research Development Foundation, Carson City, NV, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6403780	B1	20020611
APPLICATION INFO.:	US 1999-261358		19990303 (9)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 1998-201458, filed on 30 Nov 1998
DOCUMENT TYPE: Utility
FILE SEGMENT: GRANTED
PRIMARY EXAMINER: Carlson, Karen Cochrane
ASSISTANT EXAMINER: Robinson, Patricia
LEGAL REPRESENTATIVE: Adler, Benjamin Aaron
NUMBER OF CLAIMS: 12
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 13 Drawing Figure(s); 13 Drawing Page(s)
LINE COUNT: 1966

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention is directed to the cloning, sequencing and expression of homologous immunoreactive **28-kDa** protein genes, ECa28-1 and ECa28SA3, from a polymorphic multiple gene family of **Ehrlichia canis**. A complete sequence of another **28-kDa** protein gene, ECaSA2, is also provided. Further disclosed is a multigene locus encoding all five homologous **28-kDa** protein genes of **Ehrlichia canis**. Recombinant **Ehrlichia canis 28-kDa** proteins react with convalescent phase antiserum from an **E. canis**-infected dog.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

INCL INCLM: 536/023.100
INCLS: 536/023.500; 536/023.700; 536/024.200; 435/325.000; 435/252.300; 435/254.110; 435/255.100; 435/320.100; 435/326.000; 530/350.000

NCL NCLM: 536/023.100
NCLS: 435/252.300; 435/254.110; 435/255.100; 435/320.100; 435/325.000; 435/326.000; 530/350.000; 536/023.500; 536/023.700; 536/024.200

L22 ANSWER 12 OF 15 USPATFULL

ACCESSION NUMBER: 2002:126021 USPATFULL
TITLE: Ehrlichia chaffeensis 28 kDa outer membrane protein multigene family

INVENTOR(S): Walker, David H., Galveston, TX, UNITED STATES
Yu, Xue-Jie, Galveston, TX, UNITED STATES

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2002064531	A1	20020530
APPLICATION INFO.:	US 2001-846808	A1	20010501 (9)

	NUMBER	DATE
PRIORITY INFORMATION:	US 2000-201035P	20000501 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: Benjamin Aaron Adler, ADLER & ASSOCIATES, 8011 Candle Lane, Houston, TX, 77071
NUMBER OF CLAIMS: 13
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 8 Drawing Page(s)
LINE COUNT: 2104
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The 28-kDa outer membrane proteins (P28) of Ehrlichia chaffeensis are encoded by a multigene family consisting of 21 members located in a 23-kb DNA fragment in the genome of E. chaffeensis. Fifteen of these proteins are claimed herein as novel sequences. The amino acid sequence identity of the various P28 proteins was 20-83%. Six of 10 tested p28 genes were actively transcribed in cell culture grown E. chaffeensis. RT-PCR also indicated that each of the p28 genes was monocistronic. These results suggest that the p28 genes are active genes and encode polymorphic forms of the P28 proteins. The P28s were also divergent among different isolates of E. chaffeensis. The large repertoire of the p28 genes in a single ehrlichial organism and antigenic diversity of the P28 among the isolates of E. chaffeensis suggest that the P28s may be involved in immune avoidance.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
INCL INCLM: 424/190.100
INCLS: 424/234.100; 536/023.200; 435/320.100; 435/252.300; 435/189.000; 435/069.100
NCL NCLM: 424/190.100
NCLS: 424/234.100; 536/023.200; 435/320.100; 435/252.300; 435/189.000; 435/069.100

L22 ANSWER 13 OF 15 USPATFULL
ACCESSION NUMBER: 2002:116392 USPATFULL
TITLE: Homologous **28-kilodalton** immunodominant protein genes of **Ehrlicha canis** and uses thereof
INVENTOR(S): Walker, David H., Galveston, TX, United States
Yu, Xue-Jie, Houston, TX, United States
McBride, Jere W., Galveston, TX, United States
PATENT ASSIGNEE(S): Research Development Foundation, Carson City, NV, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6392023	B1	20020521
APPLICATION INFO.:	US 2000-660587		20000912 (9)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 1999-261358, filed on 3 Mar 1999 Continuation-in-part of Ser. No. US 1958-201458, filed on 30 Nov 1958
DOCUMENT TYPE: Utility
FILE SEGMENT: GRANTED
PRIMARY EXAMINER: Low, Christopher S. F.
ASSISTANT EXAMINER: Schnizer, Holly

LEGAL REPRESENTATIVE: Adler, Benjamin Aaron
NUMBER OF CLAIMS: 11
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 17 Drawing Figure(s); 20 Drawing Page(s)
LINE COUNT: 1266
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention is directed to the cloning, sequencing and expression of homologous immunoreactive **28-kDa** protein genes, **p28**-1, -2, -3, -5, -6, -7, -9, from a polymorphic multiple gene family of **Ehrlichia canis**. Further disclosed is a multigene locus encoding all nine homologous **28-kDa** protein genes of **Ehrlichia canis**. Recombinant **Ehrlichia canis 28-kDa** proteins react with convalescent phase antiserum from an **E. canis** -infected dog, and may be useful in the development of vaccines and serodiagnostics that are particularly effective for disease prevention and serodiagnosis.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
INCL INCLM: 536/023.100
INCLS: 435/320.100; 435/325.000; 435/352.300; 435/069.100
NCL NCLM: 536/023.100
NCLS: 435/069.100; 435/252.300; 435/320.100; 435/325.000

L22 ANSWER 14 OF 15 USPATFULL
ACCESSION NUMBER: 2002:51096 USPATFULL
TITLE: P43 antigen for the immunodiagnosis of canine ehrlichiosis and uses thereof
INVENTOR(S): Walker, David H., Galveston, TX, United States
McBride, Jere W., Galveston, TX, United States
PATENT ASSIGNEE(S): Research Development Foundation, Carson City, NV, United States (U.S. corporation)

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 6355777	B1	20020312	
APPLICATION INFO.:	US 2000-561322		20000428	(9)

DOCUMENT TYPE: Utility
FILE SEGMENT: GRANTED
PRIMARY EXAMINER: Swartz, Rodney P.
LEGAL REPRESENTATIVE: Adler, Benjamin Aaron
NUMBER OF CLAIMS: 6
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 7 Drawing Figure(s); 7 Drawing Page(s)
LINE COUNT: 1243
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Canine monocytic ehrlichiosis, caused by Ehrilichia canis is a potentially fatal disease of dogs that requires rapid and accurate diagnosis in order to initiate appropriate therapy leading to a favorable prognosis. In the invention described herein, a new immunoreactive E. canis surface protein gene of 1170-bp was cloned, which encodes a protein with a predicted molecular mass of 42.6 kilodaltons (P43). The P43 gene was not found in E. chaffeensis DNA by Southern blot, and antisera against recombinant P43 (rP43) did not react with E. chaffeensis by IFA. The P43 was located on the surface of E. canis by immunoelectron microscopy. Forty-two dogs exhibiting signs and/or hematologic abnormalities

associated with canine ehrlichiosis were tested by IFA and by Western immunoblot. Among the 22 samples that were IFA positive for E. canis, 100% reacted with the rP43, 96% with the rP28, and 96% with the rP140. The specificity of the recombinant proteins compared to IFA was 96% for rP28, 88% for P43 and 63% for P140. Results of this study demonstrate that the rP43 and rP28 are sensitive and reliable serodiagnostic antigens for the diagnosis of Ehrlichia canis infections.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
INCL INCLM: 530/350.000
INCLS: 424/184.100; 424/185.100; 424/190.100; 424/191.100; 424/192.100; 424/206.100; 424/234.100; 424/265.100; 435/041.000; 435/070.100; 435/071.100; 435/091.100; 530/300.000; 536/023.100; 536/023.700
NCL NCLM: 530/350.000
NCLS: 424/184.100; 424/185.100; 424/190.100; 424/191.100; 424/192.100; 424/206.100; 424/234.100; 424/265.100; 435/041.000; 435/070.100; 435/071.100; 435/091.100; 530/300.000; 536/023.100; 536/023.700

L22 ANSWER 15 OF 15 USPATFULL
ACCESSION NUMBER: 2001:97895 USPATFULL
TITLE: Nucleic acid vaccines for ehrlichia chaffeensis and methods of use
INVENTOR(S): Barbet, Anthony F., Archer, FL, United States
Ganta, Roman Reddy, Manhattan, KS, United States
McGuire, Travis C., Pullman, WA, United States
Burridge, Michael J., Gainesville, FL, United States
Nyika, Aceme, Harare, Zimbawe
Rurangirwa, Fred R., Pullman, WA, United States
Mahan, Suman M., Harare, Zimbawe
Bowie, Michael V., Gainesville, FL, United States
Alleman, Arthur Rick, Alachua, FL, United States
PATENT ASSIGNEE(S): University of Florida, Gainesville, FL, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6251872	B1	20010626
APPLICATION INFO.:	US 1997-953326		19971017 (8)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 1996-733230, filed on 17 Oct 1996, now patented, Pat. No. US 6025338
DOCUMENT TYPE: Utility
FILE SEGMENT: GRANTED
PRIMARY EXAMINER: Duffy, Patricia A.
LEGAL REPRESENTATIVE: Saliwanchik, Lloyd & Saliwanchik
NUMBER OF CLAIMS: 9
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 8 Drawing Figure(s); 8 Drawing Page(s)
LINE COUNT: 626
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Described are nucleric acid vaccines containing genes to protect animals or humans against Ehrlichia chaffeensis. Also described are polypeptides and methods of using these polypeptides to detect antibodies to pathogens.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
INCL INCLM: 514/044.000
INCLS: 435/320.100; 536/023.700
NCL NCLM: 514/044.000
NCLS: 435/320.100; 536/023.700

- Author(s)

L23 6 S L16
L24 14 S L17
L25 6 S L18
L26 12 S (L13 OR L14 OR L15) AND L1
L27 5 S (L23 OR L24 OR L25 OR L26) NOT L22

L27 ANSWER 1 OF 5 USPATFULL
ACCESSION NUMBER: 2003:134002 USPATFULL
TITLE: Ehrlichia disulfide bond formation proteins and uses thereof
INVENTOR(S): **Walker, David H.**, Galveston, TX, UNITED STATES
McBride, Jere W., League City, TX, UNITED STATES

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2003092087	A1	20030515	
APPLICATION INFO.:	US 2002-286516	A1	20021101	(10)

	NUMBER	DATE	
PRIORITY INFORMATION:	US 2001-335611P	20011101	(60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: Benjamin Aaron Adler, ADLER & ASSOCIATES, 8011 Candle Lane, Houston, TX, 77071
NUMBER OF CLAIMS: 40
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 14 Drawing Page(s)
LINE COUNT: 1449
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Novel genes encoding homologous immunoreactive thio-disulfide oxidoreductases, or disulfide bond formation (Dsb) proteins from Ehrlichia chaffeensis and **Ehrlichia canis** are disclosed. While the E. chaffeensis and **E. canis** Dsb proteins are at most only 31% or less homologous to other known Dsb proteins, the Ehrlichia Dsbs contain a cysteine active site, Cys-Gly-Tyr-Cys, similar to those in known Dsb proteins. As predicted by 15-amino acid identical N-terminal signal peptides, the proteins are primarily localized in the periplasm of E. chaffeensis and **E. canis**, possibly playing a role in antigenicity and pathogenesis. The present invention provides the nucleotide and amino acid sequences and expression vectors for the E. chaffeensis and **E. canis** dsb genes, antisera directed against the proteins, and kits to determine whether an individual or animal is infected with a given species of Ehrlichia.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
INCL INCLM: 435/007.320

Searcher : Shears 308-4994

INCLS: 435/069.300; 435/191.000; 435/320.100; 435/252.300; 536/023.700
NCL NCLM: 435/007.320
NCLS: 435/069.300; 435/191.000; 435/320.100; 435/252.300; 536/023.700

L27 ANSWER 2 OF 5 USPATFULL
ACCESSION NUMBER: 2000:37642 USPATFULL
TITLE: **Ehrlichia canis** 120-kDa immunodominant antigenic protein and gene
INVENTOR(S): **Yu, Xue-Jie**, Galveston, TX, United States
Walker, David H., Galveston, TX, United States
PATENT ASSIGNEE(S): Research Development Foundation, Carson City, NV, United States (U.S. corporation)

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 6043085		20000328	
APPLICATION INFO.:	US 1998-141047		19980827	(9)

DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Caputa, Anthony C.
ASSISTANT EXAMINER: Lee, Li
LEGAL REPRESENTATIVE: Adler, Benjamin Aaron
NUMBER OF CLAIMS: 5
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 16 Drawing Figure(s); 13 Drawing Page(s)
LINE COUNT: 1550
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention provides a 120-kDa protein gene of **Ehrlichia canis**, amplified by PCR using primers derived from the DNA sequences flanking the Ehrlichia chaffeensis 120-kDa protein gene. The recombinant **E. canis** 120-kDa protein contains 14 tandem repeat units with 36 amino acids each. The repeat units are hydrophilic and predicted to be surface-exposed. Also disclosed is that the recombinant **E. canis** 120-kDa protein is antigenic and reacts with sera from dogs convalescent from canine ehrlichiosis.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
INCL INCLM: 435/325.000
INCLS: 435/069.100; 435/070.100; 435/071.100; 435/071.200; 435/320.100; 435/455.000; 435/326.000; 435/410.000; 435/252.300; 435/254.110; 435/252.330; 435/255.100; 435/362.000; 530/350.000; 536/023.100; 536/023.500; 536/023.700; 536/024.200
NCL NCLM: 435/325.000
NCLS: 435/069.100; 435/070.100; 435/071.100; 435/071.200; 435/252.300; 435/252.330; 435/254.110; 435/255.100; 435/320.100; 435/326.000; 435/362.000; 435/410.000; 435/455.000; 530/350.000; 536/023.100; 536/023.500; 536/023.700; 536/024.200

L27 ANSWER 3 OF 5 USPATFULL
ACCESSION NUMBER: 2000:7192 USPATFULL
TITLE: Immunodominant 120 kDa surface-exposed adhesion

protein genes of Ehrlichia chaffeensis

INVENTOR(S): **Walker, David H.**, Galveston, TX, United States
Yu, Xue-Jie, Galveston, TX, United States
PATENT ASSIGNEE(S): Research Development Foundation, Carson, NV, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6015691		20000118
APPLICATION INFO.:	US 1996-656034		19960531 (8)

DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Hutzell, Paula K.
ASSISTANT EXAMINER: Masood, Khalzd
LEGAL REPRESENTATIVE: Arnold White & Durkee
NUMBER OF CLAIMS: 25
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 2 Drawing Figure(s); 2 Drawing Page(s)
LINE COUNT: 1890
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Disclosed is an isolated gene encoding a 120 kDa immunodominant antigen of Ehrlichia chaffeensis. The 120-kDa protein is one of the immunodominant proteins of E. chaffeensis that stimulates production of specific antibodies in infected humans. Also disclosed are the amino acid sequence of the 120 kDa antigen. Methods of producing a recombinant 120 kDa antigen and therapeutic methods of use of the antigen are also disclosed.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
INCL INCLM: 435/069.100
INCLS: 435/069.700; 435/252.300; 435/320.100; 530/350.000; 530/387.100; 536/023.100; 536/023.500; 536/023.400; 536/024.330; 536/024.310; 536/024.320; 536/024.300; 536/024.500; 424/234.100
NCL NCLM: 435/069.100
NCLS: 424/234.100; 435/069.700; 435/252.300; 435/320.100; 530/350.000; 530/387.100; 536/023.100; 536/023.400; 536/023.500; 536/024.300; 536/024.310; 536/024.320; 536/024.330; 536/024.500

L27 ANSWER 4 OF 5 USPATFULL

ACCESSION NUMBER: 96:94273 USPATFULL
TITLE: Gel-based vapor extractor and methods

INVENTOR(S): **Walker, David H.**, Winchester, MA, United States
Gold, Harris, Lexington, MA, United States
McKinney, III, George W., Chestnut Hill, MA, United States
McCoy, III, John F., North Chelmsford, MA, United States
Yu, Xiaohong, Boston, MA, United States
PATENT ASSIGNEE(S): Gel Sciences, Inc., Bedford, MA, United States (U.S. corporation)

	NUMBER	KIND	DATE

PATENT INFORMATION: US 5565139 19961015
APPLICATION INFO.: US 1995-459307 19950602 (8)
RELATED APPLN. INFO.: Division of Ser. No. US 1993-168723, filed on 15 Dec 1993
DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Gibson, Sharon
ASSISTANT EXAMINER: Fee, Valerie D.
LEGAL REPRESENTATIVE: Choate, Hall & Stewart
NUMBER OF CLAIMS: 18
EXEMPLARY CLAIM: 2
NUMBER OF DRAWINGS: 13 Drawing Figure(s); 9 Drawing Page(s)
LINE COUNT: 1660
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A vapor extraction apparatus includes a gel sorbent capable of absorbing vapor directly into the liquid state and capable of disgorging the absorbed liquid in a phase-transition. The apparatus includes a housing adapted for movement from a first position, where it is exposed to a vapor-containing gas stream and a first environmental condition, and capable of moving to a second position, where it is exposed to a second environmental condition. A gel sorbent is disposed on at least one surface of the housing. The gel sorbs vapor from the gas stream as liquid when the sorbent is in its first position. The sorbent disgorges the liquid during phase-transition collapse when it is in the second position. A method of extracting vapor from a process gas stream includes contacting a phase transition gel sorbent with vapor under conditions sufficient for the gel sorbent to undergo a phase transition and absorb vapor as liquid inside the gel sorbent. The gel sorbent is then exposed to conditions sufficient for it to undergo a phase transition and disgorge the liquid from inside the gel sorbent. The disgorged liquid is removed from the gel sorbent.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
INCL INCLM: 252/194.000
INCLS: 096/118.000; 096/119.000; 096/120.000; 096/125.000; 502/401.000; 502/402.000
NCL NCLM: 252/194.000
NCLS: 096/118.000; 096/119.000; 096/120.000; 096/125.000; 502/401.000; 502/402.000

L27 ANSWER 5 OF 5 USPATFULL
ACCESSION NUMBER: 96:60375 USPATFULL
TITLE: Gel-based vapor extractor and methods
INVENTOR(S): **Walker, David H.**, Winchester, MA, United States
Gold, Harris, Lexington, MA, United States
McKinney, III, George W., Chestnut Hill, MA, United States
McCoy, III, John F., North Chelmsford, MA, United States
Yu, Xiaohong, Boston, MA, United States
PATENT ASSIGNEE(S): Gel Sciences, Inc., Bedford, MA, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 5534186		19960709

APPLICATION INFO.: US 1993-168723 19931215 (8)
DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Geist, Gary
ASSISTANT EXAMINER: Fee, Valerie
LEGAL REPRESENTATIVE: Choate, Hall & Stewart
NUMBER OF CLAIMS: 33
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 13 Drawing Figure(s); 9 Drawing Page(s)
LINE COUNT: 1699
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A vapor extraction apparatus includes a gel sorbent capable of absorbing vapor directly into the liquid state and capable of disgorging the absorbed liquid in a phase-transition. The apparatus includes a housing adapted for movement from a first position, where it is exposed to a vapor-containing gas stream and a first environmental condition, and capable of moving to a second position, where it is exposed to a second environmental condition. A gel sorbent is disposed on at least one surface of the housing. The gel sorbs vapor from the gas stream as liquid when the sorbent is in its first position. The sorbent disgorges the liquid during phase-transition collapse when it is in the second position. A method of extracting vapor from a process gas stream includes contacting a phase transition gel sorbent with vapor under conditions sufficient for the gel sorbent to undergo a phase transition and absorb vapor as liquid inside the gel sorbent. The gel sorbent is then exposed to conditions sufficient for it to undergo a phase transition and disgorge the liquid from inside the gel sorbent. The disgorged liquid is removed from the gel sorbent.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
INCL INCLM: 252/194.000
INCLS: 502/401.000; 502/402.000; 502/056.000; 096/118.000; 096/119.000; 096/120.000; 096/125.000; 096/131.000; 096/133.000; 096/150.000
NCL NCLM: 252/194.000
NCLS: 096/118.000; 096/119.000; 096/120.000; 096/125.000; 096/131.000; 096/133.000; 096/150.000; 502/056.000; 502/401.000; 502/402.000

=> fil hom
FILE 'HOME' ENTERED AT 12:16:16 ON 09 JUL 2003

GenCore version 5.1.6

OM protein - nucleic search, using frame_plus_p2n model

Run on: July 3, 2003, 22:38:34 ; Search time 1981 Seconds
(without alignments)
4304.453 Million cell updates/sec

Title: US-10-062-624-40
Perfect score: 1496
Sequence: 1 MNNKLKFTIINTVLVCLLSL.....AVATLNIGYFGGEIGARLTF 293

Scoring table: BLOSUM62
Xgapop 10.0 , Xgapext 0.5
Ygapop 10.0 , Ygapext 0.5
Fgapop 6.0 , Fgapext 7.0
Delop 6.0 , Delext 7.0

Searched: 2054640 seqs, 14551402878 residues

tal number of hits satisfying chosen parameters: 4109280

Minimum DB seq length: 0
Maximum DB seq length: 2000000000

Post-processing: Minimum Match 0%
Maximum Match 100%
Listing first 45 summaries

Command line parameters:
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-Q=/cgn2_1/USPTO_spool/US10062624/runat_30062003_091229_24302/app_query.fasta_1.455
-DB=GenEmbl -QFMT=fastap -SUFFIX=rge -MINMATCH=0.1 -LOOPCL=0 -LOOPEXT=0
-UNITS=bits -START=1 -END=-1 -MATRIX=blosum62 -TRANS=human40.cdi -LIST=45
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Pred. No. is the number of results predicted by chance to have a score greater than or equal to the score of the result being printed, and is derived by analysis of the total score distribution.

SUMMARIES

Result No.	Score	% Query Match	Length	DB	ID	Description
1	1496	100.0	11329	1	AF082744	AF082744 Ehrlichia
2	1496	100.0	28254	1	AF078553	AF078553 Ehrlichia
3	1072	71.7	27190	1	ECU72291	U72291 Ehrlichia c
4	1033	69.1	14759	1	AF230642	AF230642 Ehrlichia
5	494.5	33.1	6913	1	AF324792	AF324792 Ehrlichia
6	484.5	32.4	3507	1	AF125276	AF125276 Cowdria r
7	484.5	32.4	3551	1	AF125275	AF125275 Cowdria r
8	479.5	32.1	1101	1	CRU50834	U50834 Cowdria rum
9	479.5	32.1	3535	1	AF125274	AF125274 Cowdria r
10	479.5	32.1	3538	1	AF125277	AF125277 Cowdria r
11	479.5	32.1	3541	1	AF125279	AF125279 Cowdria r
12	479.5	32.1	3572	1	AF125278	AF125278 Cowdria r
13	476.5	31.9	863	1	AF355200	AF355200 Cowdria r
14	472.5	31.6	1278	1	CRU50835	U50835 Cowdria rum
15	470.5	31.5	873	1	AF368001	AF368001 Cowdria r
16	469.5	31.4	1278	1	CRU50832	U50832 Cowdria rum
17	468.5	31.3	843	6	AX042314	AX042314 Sequence
18	468.5	31.3	4683	1	AF062761	AF062761 Ehrlichia
19	467.5	31.2	1564	1	CRU49843	U49843 Cowdria rum
c 20	464	31.0	2977	1	AF068234	AF068234 Ehrlichia
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22	461.5	30.8	873	1	AY028378	AY028378 Cowdria r
23	460	30.7	1265	1	CRU50833	U50833 Cowdria rum
24	459.5	30.7	831	1	AF368000	AF368000 Cowdria r
25	459	30.7	864	6	AX042305	AX042305 Sequence
26	456.5	30.5	840	1	AF368012	AF368012 Cowdria r
27	456.5	30.5	1467	1	CRMAP1	X74250 C.ruminanti
28	456	30.5	1263	1	CRU50831	U50831 Cowdria rum
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30	452	30.2	1263	1	CRU50830	U50830 Cowdria rum
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32	451.5	30.2	1312	1	AF077734	AF077734 Ehrlichia
33	451	30.1	1280	1	AF393389	AF393389 Ehrlichia
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35	449.5	30.0	1283	1	AF077732	AF077732 Ehrlichia
36	449.5	30.0	1307	1	AF393394	AF393394 Ehrlichia
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38	449	30.0	825	1	AF368008	AF368008 Cowdria r
39	448.5	30.0	834	1	AF368010	AF368010 Cowdria r
40	447.5	29.9	1286	1	AF393393	AF393393 Ehrlichia
41	445.5	29.8	828	1	AF368014	AF368014 Cowdria r
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ALIGNMENTS

RESULT 1

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LOCUS       AF082744               11329 bp    DNA     linear   BCT 18-SEP-2000
DEFINITION  Ehrlichia canis p28 multigene locus, partial sequence.
ACCESSION   AF082744 AF168788 AF168789
VERSION     AF082744.2  GI:10181081
KEYWORDS    .
SOURCE      Ehrlichia canis.
  ORGANISM  Ehrlichia canis
            Bacteria; Proteobacteria; alpha subdivision; Rickettsiales;
            Anaplasmataceae; Ehrlichia.
REFERENCE   1  (bases 1 to 11329)
  AUTHORS   McBride,J.W., Yu,X. and Walker,D.H.
  TITLE     Molecular cloning of the gene for a conserved major immunoreactive
            28-kilodalton protein of Ehrlichia canis: a potential
            serodiagnostic antigen
  JOURNAL   Clin. Diagn. Lab. Immunol. 6 (3), 392-399 (1999)
  MEDLINE   99242757
   PUBMED   10225842
REFERENCE   2  (bases 1 to 11329)
  AUTHORS   McBride,J.W., Yu,X.J. and Walker,D.H.
  TITLE     A conserved, transcriptionally active p28 multigene locus of
            Ehrlichia canis
  JOURNAL   Gene 254 (1-2), 245-252 (2000)
  MEDLINE   20432107
   PUBMED   10974556
REFERENCE   3  (bases 1 to 11329)
  AUTHORS   McBride,J.W., Yu,X.J. and Walker,D.H.
  TITLE     Direct Submission
  JOURNAL   Submitted (07-AUG-1998) Pathology, University of Texas Medical
            Branch, 301 University Blvd., Galveston, TX 77555-0609, USA
REFERENCE   4  (bases 1 to 11329)
  AUTHORS   McBride,J.W., Yu,X.J. and Walker,D.H.
  TITLE     Direct Submission
  JOURNAL   Submitted (04-AUG-2000) Pathology, University of Texas Medical
            Branch, 301 University Blvd., Galveston, TX 77555-0609, USA
  REMARK    Sequence update by submitter
COMMENT     On Sep 18, 2000 this sequence version replaced gi:3769522.
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BASE COUNT     4065 a    1825 c    1662 g    3777 t
ORIGIN

Alignment Scores:
Pred. No.:                1.38e-116       Length:          11329
Score:                    1496.00         Matches:         293
Percent Similarity:       100.00%         Conservative:    0
Best Local Similarity:    100.00%         Mismatches:      0
Query Match:              100.00%         Indels:          0
DB:                       1               Gaps:            0

US-10-062-624-40 (1-293) x AF082744 (1-11329)

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Qy        161 ProLysGluLysValSerAsnSerIlePheHisThrValMetArgAsnAspGlyLeuSer 180
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Qy        181 IleIleSerValIleValAsnValCysTyrAspPheSerLeuAsnAsnLeuSerIleSer 200
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Qy        281 GlyTyrPheGlyGlyGluIleGlyAlaArgLeuThrPhe 293
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RESULT 2
AF078553
LOCUS       AF078553               28254 bp    DNA     linear   BCT 02-APR-2001
DEFINITION  Ehrlichia canis major outer membrane protein P30 multigene cluster
            1, complete sequence.
ACCESSION   AF078553 AF078554 AF078555 AH006958
VERSION     AF078553.2  GI:13512584
KEYWORDS    .
SOURCE      Ehrlichia canis.
  ORGANISM  Ehrlichia canis
            Bacteria; Proteobacteria; alpha subdivision; Rickettsiales;
            Anaplasmataceae; Ehrlichia.
REFERENCE   1  (bases 1 to 28254)
  AUTHORS   Ohashi,N., Unver,A., Zhi,N. and Rikihisa,Y.
  TITLE     Cloning and characterization of multigenes encoding the
            immunodominant 30-kilodalton major outer membrane proteins of
            Ehrlichia canis and application of the recombinant protein for
            serodiagnosis
  JOURNAL   J. Clin. Microbiol. 36 (9), 2671-2680 (1998)
  MEDLINE   98371112
   PUBMED   9705412
REFERENCE   2  (bases 1 to 28254)
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  AUTHORS   Ohashi,N., Rikihisa,Y. and Unver,A.
  TITLE     Analysis of transcriptionally active gene clusters of major outer
            membrane protein multigene family in Ehrlichia canis and E.
            chaffeensis
  JOURNAL   Infect. Immun. 69 (4), 2083-2091 (2001)
  MEDLINE   21153566
   PUBMED   11254561
REFERENCE   3  (bases 1 to 28254)
  AUTHORS   Ohashi,N., Unver,A., Zhi,N. and Rikihisa,Y.
  TITLE     Direct Submission
  JOURNAL   Submitted (16-JUL-1998) Department of Veterinary Biosciences, The
            Ohio State University, 1925 Coffey Road, Columbus, OH 43210, USA
REFERENCE   4  (bases 1 to 28254)
  AUTHORS   Ohashi,N., Rikihisa,Y. and Unver,A.
  TITLE     Direct Submission
  JOURNAL   Submitted (29-NOV-2000) Department of Veterinary Biosciences, The
            Ohio State University, 1925 Coffey Road, Columbus, OH 43210, USA
  REMARK    Sequence update by submitter
COMMENT     On or before Apr 2, 2001 this sequence version replaced gi:3790556,
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DEFINITION  Ehrlichia chaffeensis strain Arkansas major outer membrane protein
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ACCESSION   U72291 AF021338
VERSION     U72291.2  GI:13511827
KEYWORDS    .
SOURCE      Ehrlichia chaffeensis.
  ORGANISM  Ehrlichia chaffeensis
            Bacteria; Proteobacteria; alpha subdivision; Rickettsiales;
            Anaplasmataceae; Ehrlichia.
REFERENCE   1  (bases 14844 to 21136; 21479 to 22234)
  AUTHORS   Ohashi,N., Zhi,N., Zhang,Y. and Rikihisa,Y.
  TITLE     Immunodominant major outer membrane proteins of Ehrlichia
            chaffeensis are encoded by a polymorphic multigene family
  JOURNAL   Infect. Immun. 66 (1), 132-139 (1998)
  MEDLINE   98084465
   PUBMED   9423849
REFERENCE   2  (bases 1 to 27190)
  AUTHORS   Ohashi,N., Rikihisa,Y. and Unver,A.
  TITLE     Analysis of transcriptionally active gene clusters of major outer
            membrane protein multigene family in Ehrlichia canis and E.
            chaffeensis
  JOURNAL   Infect. Immun. 69 (4), 2083-2091 (2001)
  MEDLINE   21153566
   PUBMED   11254561
REFERENCE   3  (bases 1 to 27190)
  AUTHORS   Ohashi,N., Zhi,N., Zhang,Y. and Rikihisa,Y.
  TITLE     Direct Submission
  JOURNAL   Submitted (24-SEP-1996) Department of Veterinary Biosciences, The
            Ohio State University, 1925 Coffey Rd., Columbus, OH 43210-1093,
            USA
REFERENCE   4  (bases 1 to 27190)
  AUTHORS   Ohashi,N., Zhi,N., Zhang,Y. and Rikihisa,Y.
  TITLE     Direct Submission
  JOURNAL   Submitted (27-AUG-1997) Department of Veterinary Biosciences, The
            Ohio State University, 1925 Coffey Rd., Columbus, OH 43210-1093,
            USA
REFERENCE   5  (bases 1 to 27190)
  AUTHORS   Ohashi,N., Rikihisa,Y. and Unver,A.
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GenCore version 5.1.6

OM protein - protein search, using sw model

Run on: July 8, 2003, 10:07:40 ; Search time 72 Seconds
(without alignments)
542.256 Million cell updates/sec

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Perfect score: 1496
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Scoring table: BLOSUM62
Gapop 10.0 , Gapext 0.5

Searched: 908470 seqs, 133250620 residues

Total number of hits satisfying chosen parameters: 908470

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Post-processing: Minimum Match 0%
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Listing first 45 summaries

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Pred. No. is the number of results predicted by chance to have a score greater than or equal to the score of the result being printed, and is derived by analysis of the total score distribution.

SUMMARIES

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3	1033	69.1	291	23	AAU73409	Ehrlichia chaffeen
4	1032.5	69.0	300	23	AAU73411	Ehrlichia chaffeen
5	1026	68.6	293	23	AAU73412	Ehrlichia chaffeen
6	1006.5	67.3	298	23	AAU73410	Ehrlichia chaffeen
7	999.5	66.8	292	20	AAY06967	E. canis P30-7 pro
8	995	66.5	280	20	AAY06949	E. chafeensis OMP-
9	930	62.2	271	20	AAY06958	E. chafeensis OMP-
10	923	61.7	299	20	AAY06968	E. canis P30-8 pro
11	789	52.7	285	20	AAY06957	E. chafeensis OMP-
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13	786.5	52.6	279	20	AAY06954	E. chafeensis OMP-
14	779.5	52.1	279	23	AAU73405	Ehrlichia chaffeen
15	768	51.3	283	20	AAY06955	E. chafeensis OMP-
16	768	51.3	283	23	AAU73406	Ehrlichia chaffeen
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18	737.5	49.3	275	23	AAU73407	Ehrlichia chaffeen
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20	671	44.9	295	20	AAY06953	E. chafeensis OMP-
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22	574	38.4	164	20	AAY06951	E. chafeensis OMP-
23	519	34.7	272	23	AAU73403	Ehrlichia chaffeen
24	500.5	33.5	161	20	AAY06969	E. canis P30-9 pro
25	498	33.3	283	20	AAY06944	E. chafeensis OMP-
26	498	33.3	283	23	AAU96106	Ehrlichia chafeens
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29	470.5	31.5	280	20	AAY06948	E. chafeensis OMP-
30	470.5	31.5	280	23	AAU96110	Ehrlichia chafeens
31	470.5	31.5	288	20	AAY06959	E. canis P30 prote
32	468.5	31.3	280	19	AAW51094	Ehrlichia chaffeen
33	468.5	31.3	280	21	AAB36188	Ehrlichia chaffeen
34	468.5	31.3	280	22	AAU04198	Variable surface a
35	468.5	31.3	280	23	AAU73417	Ehrlichia chaffeen
36	464	31.0	271	23	AAU73419	Ehrlichia chaffeen
37	459	30.7	287	19	AAW51088	Cowdria ruminantiu
38	459	30.7	287	21	AAB36182	Cowdria ruminatium
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40	456.5	30.5	284	23	AAU96111	Cowdria ruminantiu
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42	450.5	30.1	280	23	AAU96107	Ehrlichia chafeens
43	450.5	30.1	280	23	AAU73414	Ehrlichia chaffeen
44	448	29.9	284	23	AAU73400	Ehrlichia chaffeen
45	446.5	29.8	280	21	AAY71479	Ehrlichia canis im

ALIGNMENTS

RESULT 1
AAY06965
ID AAY06965 standard; Protein; 293 AA.
XX
AC AAY06965;
XX
DT 05-JUL-1999 (first entry)
XX
DE E. canis P30-5 protein.
XX
KW Outer membrane protein; OMP; Ehrlichia chafeensis; E. canis; P30;
KW detection; dog.
XX
OS Ehrlichia canis.
XX
PN WO9913720-A1.
XX
PD 25-MAR-1999.
XX
PF 18-SEP-1998; 98WO-US19600.
XX
PR 19-SEP-1997; 97US-0059353.
XX
PA (OHIS) UNIV OHIO STATE.
XX
PI Ohashi N, Rikihisa Y;
XX
DR WPI; 1999-254290/21.
DR N-PSDB; AAX34765.
XX
PT Novel outer membrane proteins from Ehrlichia chaffeensis and
PT Ehrlichia canis
XX

PS Disclosure; Fig 25B; 55pp; English.
XX
CC The invention provides isolated outer membrane proteins (OMP) from
CC Ehrlichia chafeensis and E. canis. The E. chaffeensis proteins form part
CC of the OMP family and consist of proteins OMP-1, -1(B to Z) shown
CC in AAY06943-958. The E. canis proteins form part of the P30 family and
CC consist of proteins shown in AAY06959-970. The proteins and genes are
CC used to detect E. chaffeensis in patients and E. canis in dogs.
XX
SQ Sequence 293 AA;

Query Match 100.0%; Score 1496; DB 20; Length 293;
Best Local Similarity 100.0%; Pred. No. 2.3e-135;
Matches 293; Conservative 0; Mismatches 0; Indels 0; Gaps 0;

Qy 1 MNNKLKFTIINTVLVCLLSLPNISSSKAINNNAKKYYGLYISGQYKPSVSVFSNFSVKET 60
Db 1 MNNKLKFTIINTVLVCLLSLPNISSSKAINNNAKKYYGLYISGQYKPSVSVFSNFSVKET 60

Qy 61 NVITKNLIALKKDVDSIETKTDASVGISNPSNFTIPYTAVFQDNSVNFNGTIGYTFAEGT 120
Db 61 NVITKNLIALKKDVDSIETKTDASVGISNPSNFTIPYTAVFQDNSVNFNGTIGYTFAEGT 120

Qy 121 RVEIEGSYEEFDVKNPGGYTLSDAYRYFALAREMKGNSFTPKEKVSNSIFHTVMRNDGLS 180
Db 121 RVEIEGSYEEFDVKNPGGYTLSDAYRYFALAREMKGNSFTPKEKVSNSIFHTVMRNDGLS 180

Qy 181 IISVIVNVCYDFSLNNLSISPYICGGAGVDAIEFFDVLHIKFAYQSKLGIAYSLPSNISL 240
Db 181 IISVIVNVCYDFSLNNLSISPYICGGAGVDAIEFFDVLHIKFAYQSKLGIAYSLPSNISL 240

Qy 241 FASLYYHKVMGNQFKNLNVQHVAELASIPKITSAVATLNIGYFGGEIGARLTF 293
Db 241 FASLYYHKVMGNQFKNLNVQHVAELASIPKITSAVATLNIGYFGGEIGARLTF 293

RESULT 2
AAU96115
ID AAU96115 standard; Protein; 293 AA.
XX
AC AAU96115;
XX
DT 02-JUL-2002 (first entry)
XX
DE Ehrlichia canis p28-1.
XX
KW Ehrlichia canis infection; vaccine; serodiagnostic; p28;
KW antibacterial.
XX
OS Ehrlichia canis.
XX
PN WO200222782-A2.
XX
PD 21-MAR-2002.
XX
PF 12-SEP-2001; 2001WO-US28759.
XX
PR 12-SEP-2000; 2000US-0660587.
XX
PA (RERE-) RES DEV FOUND.
XX
PI Walker DH, Yu X, McBride JW;
XX
DR WPI; 2002-351882/38.
DR N-PSDB; ABK68875.
XX
PT New recombinant homologous 28 kilodalton immunodominant protein from
PT Ehrlichia canis, useful for treating Ehrlichia canis infections -
XX
PS Claim 16; Figure 13; 106pp; English.
XX
CC The invention relates to a recombinant homologous 28 kDa immunodominant
CC protein, P28, (I), of Ehrlichia canis. (I), a 28-kDa antigen preferably
CC dispersed in a pharmaceutically acceptable carrier, is useful for
CC inhibiting E. canis infection in a subject. (I) is useful in the
CC development of vaccines and serodiagnostics that are particularly
CC effective for disease prevention and serodiagnosis. AAU96100-AAU96118
CC represent the 28-kDa antigen amino acid sequences of the invention.
XX
SQ Sequence 293 AA;

Query Match 100.0%; Score 1496; DB 23; Length 293;
Best Local Similarity 100.0%; Pred. No. 2.3e-135;
Matches 293; Conservative 0; Mismatches 0; Indels 0; Gaps 0;

Qy 1 MNNKLKFTIINTVLVCLLSLPNISSSKAINNNAKKYYGLYISGQYKPSVSVFSNFSVKET 60
Db 1 MNNKLKFTIINTVLVCLLSLPNISSSKAINNNAKKYYGLYISGQYKPSVSVFSNFSVKET 60

Qy 61 NVITKNLIALKKDVDSIETKTDASVGISNPSNFTIPYTAVFQDNSVNFNGTIGYTFAEGT 120
Db 61 NVITKNLIALKKDVDSIETKTDASVGISNPSNFTIPYTAVFQDNSVNFNGTIGYTFAEGT 120

Qy 121 RVEIEGSYEEFDVKNPGGYTLSDAYRYFALAREMKGNSFTPKEKVSNSIFHTVMRNDGLS 180
Db 121 RVEIEGSYEEFDVKNPGGYTLSDAYRYFALAREMKGNSFTPKEKVSNSIFHTVMRNDGLS 180

Qy 181 IISVIVNVCYDFSLNNLSISPYICGGAGVDAIEFFDVLHIKFAYQSKLGIAYSLPSNISL 240
Db 181 IISVIVNVCYDFSLNNLSISPYICGGAGVDAIEFFDVLHIKFAYQSKLGIAYSLPSNISL 240

Qy 241 FASLYYHKVMGNQFKNLNVQHVAELASIPKITSAVATLNIGYFGGEIGARLTF 293
Db 241 FASLYYHKVMGNQFKNLNVQHVAELASIPKITSAVATLNIGYFGGEIGARLTF 293

RESULT 3
AAU73409
ID AAU73409 standard; Protein; 291 AA.
XX
AC AAU73409;
XX
DT 12-MAR-2002 (first entry)
XX
DE Ehrlichia chaffeensis outer membrane protein P28-10.
XX
KW Ehrlichia; outer membrane protein; P28; antibiotic; vaccine.
XX
OS Ehrlichia chaffeensis.
XX
PN WO200183699-A2.
XX
PD 08-NOV-2001.
XX
PF 01-MAY-2001; 2001WO-US13997.
XX
PR 01-MAY-2000; 2000US-201035P.
XX
PA (RERE-) RES DEV FOUND.
XX
PI Walker DH, Yu X;
XX
DR WPI; 2002-066527/09.
XX
PT Novel Ehrlichia chaffeensis 28-kDa outer membrane protein, designated
PT P28 useful as a vaccine against Ehrlichia chaffeensis -
XX
PS Claim 10; Figure 2; 97pp; English.
XX
CC The invention relates to isolated and purified 28-kDa outer membrane
CC proteins (P28-1 to P28-21) of Ehrlichia chaffeensis. P28 proteins
CC are encoded by a 28kDa outer membrane protein multigene family. P28
CC proteins are useful as a vaccine against E.chaffeensis. DNA encoding P28
CC is useful for transfecting a host cell. AAU73400-AAU73420 represent
CC Ehrlichia chaffeensis P28 outer membrane proteins of the invention.
XX